How Do Rates of Carbon Metabolism Vary over a Geological Gradient, and How does this Contribute to Riverine Greenhouse Gas Emissions?

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Submitted in partial fulfilment of the requirements of the Degree of Doctor of Philosophy

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Abstract

Rivers and streams are increasingly recognised as important components in the global carbon cycle, and act as net sources of CO_2 and CH_4 to the atmosphere. However, the origins and controls over the fate of these greenhouse gases are still poorly constrained. This thesis firstly explores the production of CO_2 and CH_4 in the sediment of several rivers over a geological gradient (chalk, sand and clay), to investigate the magnitude and controls on production. It was found that, whilst there are some general patterns due to geology, variables such as organic carbon content are much better predictors of production of CO_2 and CH_4 and these can vary widely within a single reach. The response of production to temperature was found to be very constant across rivers and in both summer and winter, suggesting a uniform increase in production of both CO_2 and CH_4 with projected future climate change. However, production of CH_4 was much more responsive to temperature change than was CO_2, meaning a greater proportion of carbon is mineralized as CH_4 under warmer conditions and indicating a positive feedback with global warming.

In addition, the amount of CO_2 and CH_4 out-gassed from the rivers to the atmosphere was measured. It was found that the amount out-gassed could not be explained by local sediment respiration alone: CO_2 out-gassing was consistently higher (and CH_4 consistently lower) than that produced by the riverbed. Instead, CO_2 out-gassing was under hydrological control, and was correlated with rainfall. The source of this was ingress from the surrounding catchment, with higher out-gassing during periods of high rainfall. This strong hydrological controls on CO_2 emissions were however modulated by biological processes, as lower emissions were measured during the day than night; and the difference could be accounted for by local in-stream GPP.

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1. Introduction

1.1 Rivers and the carbon cycle

The global carbon cycle is the process by which carbon is cycled through the land, oceans and atmosphere; by physical, chemical and biological processes. In recent decades this cycle has been disturbed by anthropogenic activities, particularly burning of fossil fuels and land use changes. As such, it is of particular importance to be able to describe the carbon cycle in detail, and identify the various reservoirs of carbon and the fluxes between them; and to predict future changes.

Freshwater systems such as rivers, streams and lakes have been increasingly studied with respect to carbon cycling, and current understanding shows that they are a significant component of the global carbon cycle (Battin*et al*. 2009; Tranvik *et al*. 2009; Aufdenkampe *et al*. 2011; Cole *et al*. 2007). Freshwaters are a link between terrestrial and oceanic ecosystems: large amounts of carbon in terrestrial systems are carried by lateral transport to freshwaters (Schlesinger & Melack 1981). The carbon transported to freshwaters is a combination of both organic carbon, formed by terrestrial primary production; and inorganic carbon compounds such as carbon dioxide (CO_2) and methane (CH_4), originating from chemical weathering as well as mineralisation by terrestrial heterotrophs.

Until recently, models of the global carbon cycled represented rivers and other freshwaters as simply a passive 'pump', carrying this carbon from the terrestrial systems it originates in to the oceans (Fischlin *et al.* 2007). It is then incorporated into wider oceanic carbon cycle estimates. However, it is now apparent that freshwaters are active sites of metabolism, and are responsible for chemically and biologically transforming, storing and out-gassing carbon as well as transporting a portion to the oceans (Battin *et al*. 2009). Around 30-80% of organic carbon lost from terrestrial systems is estimated to be mineralised in freshwater systems (Algesten *et al.* 2003). Therefore, although they only cover 0.30-0.56% of the global land surface (Downing *et al.* 2012) rivers and other freshwaters are now recognised

as an important component of the global carbon cycle, more significant than their small global surface area would suggest.

Current estimates propose that rivers receive 2.9 PgC yr^{-1} from terrestrial export and rock weathering. But they only deliver 0.9 PgC yr^{-1} to the oceans. Most of the rest (1.4 PgC yr^{-1}) is out-gassed to the atmosphere, with the remainder (0.6 PgC yr^{-1}) buried in freshwater sediments (Tranvik *et al.* 2009). Evidently the amount of carbon out-gassed by freshwaters is of a significant magnitude, similar to total terrestrial NEP (Cole *et al.* 2007); and so the mechanisms and controls of this need to be fully elucidated. Estimates of local NEP often do not extrapolate well to larger scales, and one source of this error may be neglecting to consider lateral transport to freshwaters (Aufdenkampe *et al.* 2011).

The organic fraction of carbon can be mineralised by biological (respiration) and chemical (photochemical degradation) in-stream processes. The main site of biological metabolism is microbial communities in the sediment. There is evidence that organic carbon which would be considered recalcitrant on land and part of the stored carbon reservoir can be readily mineralised when it reaches freshwaters (Cole & Caraco 2001; Lapierre *et al.* 2013). Additionally, due to the higher occurrence of anoxia in waterlogged sediments, a higher fraction of organic carbon may be mineralised to CH_4 rather than CO_2 (Cole *et al.* 2007) compared to on land, as methanogenesis occurs in highly anaerobic conditions. This suggests that the fate of carbon compounds in freshwater systems are not necessarily the same as on land; so, these systems need to be considered separately from terrestrial or oceanic systems, with accurate carbon budgets.

It is clear therefore that understanding and incorporating freshwaters into models of carbon cycling is necessary, and can have a significant effect on predictions of carbon cycling. In order to properly incorporate freshwaters, more needs to be understood about the delivery of carbon to these systems as well as the processes occurring within them, what the controlling factors are, as well as changes due to projected climate change and land use

change. Climate change will have an effect on precipitation, with more extreme events including floods and droughts becoming more common (IPCC 2013). This will affect the delivery of both water and dissolved components to freshwaters from surrounding terrestrial catchments, as well as flow rate (Kundzewicz *et al.* 2008). Furthermore increases in atmospheric CO_2 concentrations may suppress transpiration, causing a subsequent increase in runoff (Gedney *et al.* 2006). In addition, increasing temperature and changes in seasonal patterns may affect the microbial communities in freshwater sediments, with accompanying alterations to carbon metabolism.

1.2 Carbon metabolism in freshwaters

Due to the input of carbon from surrounding terrestrial systems, rivers and other freshwater systems are usually supersaturated with CO_2 and CH_4; and as such they are net sources of these gases to the atmosphere (Hope *et al.* 2001; Raymond & Cole 2001; Butman & Raymond 2011; Raymond *et al.* 2013). Additionally, because a portion of the carbon input is labile organic matter, they are often net heterotrophic, i.e. rates of respiration exceed primary production (Bunn *et al.* 2003; Acuña *et al.* 2004; Houser *et al.* 2005; Duarte & Prairie 2005). This contrasts with most systems in terrestrial spheres, where respiration cannot exceed the rate of production of organic compounds by local autotrophs.

Whilst this carbon subsidy is large, with an estimated 40-55% of particulate organic carbon in freshwaters being terrestrial in origin (Pace *et al.* 2004), it is important to note as well the presence of autochthonous organic carbon, formed by primary production *in situ* by autotrophic organisms. These include submerged vegetation, attached algae (periphyton) and single celled photosynthetic organisms. The prevalence of these primary producers in a system depends on physical factors such as the availability of sunlight, oxygen and nutrients including nitrogen and phosphate, pH, water depth and flow rate (Alvarez-Cobelas *et al.* 2010). Consequently, the rate of primary production, as well as carbon mineralisation by respiration and methanogenesis, should be expected to vary across different freshwater habitats as well as seasonally and geographically.

As well as metabolism of carbon, the atmospheric exchange of CO_2 and CH_4 needs to be considered. Since freshwaters are generally supersaturated with CO_2 and CH_4 compared to the atmosphere, there is normally a net flux from the water to the air, and there are higher emissions from streams compared to surrounding terrestrial systems (Crawford *et al.* 2014). As well as the concentration gradient, fluxes are affected by physical factors including wind speed and water current (Beaulieu *et al.* 2012); and can vary spatially and temporally (Casper *et al.* 2000). CO_2 is a highly soluble gas, so is out-gassed predominantly by diffusion. But CH_4 is not

very water soluble, so a significant proportion is released sporadically in bubbles. This makes point measurements inaccurate (Casper *et al.* 2000; Beaulieu *et al.* 2012).

1.3 Stream and riverine ecosystems

Whilst there are several estimates of freshwater carbon fluxes on a global scale, these do not reflect adequately the range and diversity of freshwater systems. There are published estimates of more than 80 of the world's largest rivers (Cole & Caraco 2001), as well as many lakes and estuaries. But the differences between ecosystems even at a local scale may be expected to bring about considerable variation in carbon cycling. In particular, small streams represent a "major unknown" (Cole *et al.* 2007), as there is a lack of estimates of carbon metabolism in these systems. According to the River Continuum Concept, changes in organic matter, metabolism and community structure are expected along fluvial systems from headwaters to estuaries, in response to changes in physical structures and conditions (Vannote *et al*. 1980; Battin *et al.* 2009). Consequently, including the full range of freshwaters is vital in order to calculate accurate values of metabolism.

Base flow index (BFI) is a measure of what proportion of a river's total water is contributed by groundwater. Rivers and streams with a high BFI are those which most of their water comes from groundwater and the flow rate and river level is usually very stable. Conversely rivers and streams with a low BFI do not receive a large proportion of their water from groundwater: instead they are more dependent on rain water input. These rivers are characterised by more variation in depth, with flow rates that vary widely depending on recent and seasonal weather patterns. Smaller, headwater streams in particular are strongly influenced by local weather patterns.

The BFI of a particular river depends in part on its surrounding geology (Bloomfield *et al.* 2009). Rivers on permeable geology such as chalk will typically have high BFIs and a large groundwater input. Rivers on impermeable, clay catchments will have low BFIs and a high rainwater input. Intermediate, sandy geologies have BFI values that are between the two. Chalk rivers tend to have high nutrient levels from the input of groundwater, including inorganic carbon compounds from rock weathering (Hope *et al.* 1994). Rainwater contains few

impurities, but solutes and suspended matter including organic carbon are added to it as it flows over land and into rivers and streams (Dobson & Frid 2009). Thus BFI and geology influence concentrations and availability of dissolved nutrients and other components in river water.

As well as BFI, other differences are apparent between geological landscapes. The clear water typical in chalk streams allows light to penetrate further, and they often have considerable amounts of vegetation growth (Clarke 2002). Macrophytes, such as *Ranunculus spp.*, are common, as well as algae and single celled photosynthetic organisms. This suggests that, whilst they do not have the high terrestrial organic matter input that rivers with low BFI values have, the inorganic carbon from groundwater together with the presence of primary producers suggests a large amount of autochthonous organic carbon. This would then be available for respiration and methanogenesis by heterotrophic organisms present in the sediment. Conversely, rivers with low BFIs may not have substantial autochthonous carbon fixation, but could have a higher subsidy of allochthonous (i.e. terrestrial) organic carbon input. In most riverine environments the input of allochthonous organic carbon exceeds that produced by local autotrophs (Cole & Caraco 2001).

Macrophytes are also important as they provide structural complexity and heterogeneity, causing occurrence of different microhabitats (Tokeshi & Pinder 1985), and oxygen translocation to the roots changes the chemistry and redox state of the surrounding sediment (Moore *et al*1994*.,* Clarke 2002). High densities of macrophytes decrease flow velocity, which traps fine sediments (Dawson 1981), and display seasonal patterns, with maximum biomass during summer and dieback occurring during autumn (Clarke 2002).

Other considerations when comparing rivers are characteristics of the surrounding terrestrial area, such as tree cover. When rivers are tree-lined, a substantial amount of leaf litter can fall into the water, and represents a major input of allochthonous carbon during autumn (Webster & Benfield 1986). Additionally, tree cover causes a reduction in light

penetration. Also, how the land is being used in the surrounding catchment may alter metabolism in a river, for example agricultural land may deliver large quantities of nitrogen and phosphorus, nutrients that are often limiting to biological processes in ecosystems.

Since factors including temperature, vegetation, leaf fall and light penetration are expected to be important in carbon cycling in rivers, season is an important consideration. All these things will be affected by seasonal changes and as such need to be included in comprehensive estimates of carbon cycling in rivers. As well as the metabolism, physical factors such as flow rate and chemical factors such as photochemical degradation may affect out-gassing of both locally produced carbon gases and lateral transport of gases with terrestrial origins. As well as the yearly seasonal cycle, the 24-hour diel cycle will affect carbon metabolism so measurements need to consider this. It is well established that respiration and CO_2 production is modulated by photosynthesis in the daylight, but there are also suggested influences of light on net methanogenesis (King 1990; Dumestre *et al.* 1999; Podgrajsek *et al.* 2014).

The emissions of CO_2 and CH_4 also need to be considered with respect to the properties of the stream. Turbulence caused by macrophytes (Clarke 2002) or other obstructions will increase the rate of out-gassing (Bade 2010). Headwater streams are more connected to their terrestrial catchment than larger rivers which get a greater proportion of their water from upstream, and so can be more affected by seasonal conditions and weather events.

1.4 Conclusion

This introductory section has shown that freshwaters such as rivers and streams are important locations of carbon cycling, and neglecting them in wider carbon modelling results in incorrect assumptions and oversimplifications. Fittingly, there have recently been many efforts to incorporate these ecosystems in estimates of carbon fluxes, sources and sinks (Battin*et al.* 2009; Cole *et al.* 2007; Fischlin *et al.* 2007). However, small streams are under-represented and these are complex systems which are the site of both local metabolic cycles and the transport and transformation of carbon compounds from the much larger terrestrial systems that are their catchments. Consequently it is necessary to identify and explain the metabolism and out-gassing of carbon in these systems with respect to geological catchment and other river properties; as well as seasonal and diel cycles. This way, scaled-up estimates can be calculated with more detailed constraints on how different systems may behave differently. Additionally, proper investigation of how changes in temperature and sediment properties alter carbon metabolism allows predictions with respect to future global warming and land use change to be made. Finally, many studies in the literature focus on measurements of pCO_2, relying on several assumptions to estimate fluxes and local production rates, and often do not include CH_4 contributions. These measurements may not incorporate fully the effects of diel or seasonal changes, or be able to accurately describe the connection between lateral input, local metabolism and atmospheric exchanges.

1.5 Scope of Thesis

This thesis will consider the production of CO_2 and CH_4 in river sediments as well as the out-gassing of these gases from the river to the atmosphere. Experiments were carried out across several rivers in a catchment in Southern England, such that a range of geology types and hence base flow indexes could be included. Laboratory incubations were carried out, to quantify potential production of CO_2 and CH_4 across a temperature gradient. *In situ* experiments were also performed, to find the production of CO_2 and CH_4 during light and dark, as well as concurrent measurements of the flux of CO_2 and CH_4 to the atmosphere. These detailed, direct measurements will then be used to understand how the relationship between terrestrial and riverine ecosystems varies with geology and season, as well as how geology, season, temperature and diel cycles affect the production of CO_2 and CH_4 in river sediments. Since measurements of production and out-gassing are performed concurrently, the contribution of local metabolism to out-gassing can be directly calculated.

Thus the research carried out for this thesis will contribute new information about the place of rivers in the carbon cycle, and illustrate the connections between land and river, as well as between local production and atmospheric out-gassing. This will provide responses to the hitherto unanswered questions of How do rates of carbon metabolism vary over a geological gradient? and How does this contribute to riverine greenhouse gas emissions?

1.6 Key Questions

Overall Question:

What rates of carbon metabolism are found in rivers over a geological gradient, and how do they contribute to riverine greenhouse gas emissions?

Chapter 2:	**Chapter 3:**	**Chapter 4:**
What is the potential for CO_2 and CH_4 production across the sites at different temperatures, and what could expain any differences?	What is the actual metabolism of the sites in situ, factoring in diel, seasonal and patch variation, and how does this change with geology?	How much CO_2 and CH_4 are out-gassed by the rivers, and what proportion of this is produced by in situ metabolism?

Conclusion:

How the data chapters together answer the overall question, and the implications of this work.

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2. Study Site and Experimental Approach

2.1 NERC Macronutrient Cycles Programme

This PhD project was carried out as part of the macronutrient cycles programme, funded by the Natural Environment Research Council (NERC). The overall aim of this programme was to quantify the scales of nitrogen (N) and phosphorus (P) fluxes and transformations throughout a catchment, and relate it to the carbon cycle (Macronutrient Cycles Programme 2016). More specifically, this PhD was within the consortium project named 'The role of lateral exchange in modulating the seaward flux of C, N, P'. This project took place on the Hampshire Avon, and the central hypothesis was that underlying geology, which could be expressed by base flow index (BFI), would have a crucial role in carbon flux and metabolism, and so regulate flux and dynamics of N and P.

2.2 The Hampshire Avon

The study site chosen for this project was headwater tributaries of the Hampshire Avon, in Dorset and Wiltshire, UK (Figure 2.1). The Hampshire Avon catchment covers a total area of 1750 km^2(Environment Agency 2016) and lies predominantly on permeable Cretaceous chalk. However there tributaries in the north of the catchment (West and East Avon) are on a less permeable, greensand geology; and under tributaries in the west (Sem and Nadder) there is significant Cretaceous and Jurassic impermeable clay (Jarvie *et al.* 2008). The land use of the catchment is predominantly rural (98%), composed of arable fields, pastured grasslands (mostly dairy farming) and woodlands (DTC 2010).

The catchment was chosen for the wider consortium project due to its headwater streams being located on a range of geologies (chalk, sand and clay) in order to give a full range of BFI (Figure 2.2). In total, there were nine streams used in the study site (three of each chalk, sand and clay), as shown in Table 2.1, though only 6 of these were used for the longer field campaigns and *in situ* measurements (two of each geology).

Thesample sites in thestudy catchments are shown with underlying geology in Figure 2.2. Due to the difficulty in finding sites of the appropriate size, geology and getting permission to work on them; some of the sample sites are close to each other or are tributaries of other sample sites, rather than being truly independent catchments.Rushall-Sand (sand) is a tributary of the Avon (sand) sample site. Cools and Priors (both clay) are tributaries of the Sem (clay). The Sem and Nadder (sand) sample sites are on the same farm. These rivers converge within a few hundred metres of the sample sites. All other sites have sources independent of other sampling sites, and converge much further down the river network.

Fieldwork and data collection were carried out between February 2013 and June 2015. The average rainfall for the region (1910-1915) is shown in Figure 2.3 (grey bars). During the field campaign period, extreme weather patterns were seen. Whilst the rainfall was near average initially, the summer period was characterised by rainfall approximately half the average.

However this switched after September, with October 2013 to February 2014 experiencing some of the highest rainfall on record. This affected the research, as heavy flooding during the autumn and winter meant some rivers were inaccessible, so experiments could not be carried out. However, it does mean that the sampling that was carried out was done over a large gradient of weather conditions, from very low rainfall to flooding conditions. This had a resultant effect on discharge and water level, with considerable difference between the seasons.

River	Geology	BFI	BFI-HOST	Stream Order	Catchment Size (km^2)	Land use
Ebble	Chalk (96%)	0.905	0.931	1 (28%) 2 (72%)	58.9	Arable (55%), Grassland (50%)
Wylye	Chalk (80%)	0.885	0.953	1 (60%) 2 (40%)	53.5	Arable (50%), Grassland (35%)
Rushall-Chalk	Chalk					
Avon	Sand (50%)	0.732	0.861	1 (47%) 2 (31%) 3 (22%)	59.2	Arable (25%), Grassland (50%)
Rushall-Sand	Sand					
Nadder	Sand (52%)	0.814	0.695	1 (58%) 2 (39%) 3 (3%)	34.6	Arable (46%), Grassland (33%)
Sem	Clay (74%)	0.207	0.234	1 (54%) 2 (26%) 3 (20%)	26.0	Arable (10%), Grassland (90%)
Priors	Clay (>99%)	0.551	0.372	1 (73%) 2 (18%) 3 (9%)	4.9	Arable (5%), Grassland (95%)
Cools	Clay					

Table 2.1: Summary of characteristics for each of the rivers in the study. Base Flow Index (BFI) is derived from a hydrograph (Gustard *et al.*1992), and is a measure of the proportion of river runoff that comes from groundwater input, correlated with catchment geology. Chalk streams, due to the high permeability of the rock, have high BFI values whilst rivers on impermeable geology have a greater proportion of water input from rainwater, and thus lower BFI values. BFI here are calculated from discharge data collected from July 2013-2014. BFI-HOST is based on the soil type of the catchment and calculated using the UK Hydrology of Soil Types (HOST) classification. Strahler stream order with % contribution of stream order to the network. Major land use based on 2010 agcensus data. Table from Heppell et al (unpublished).

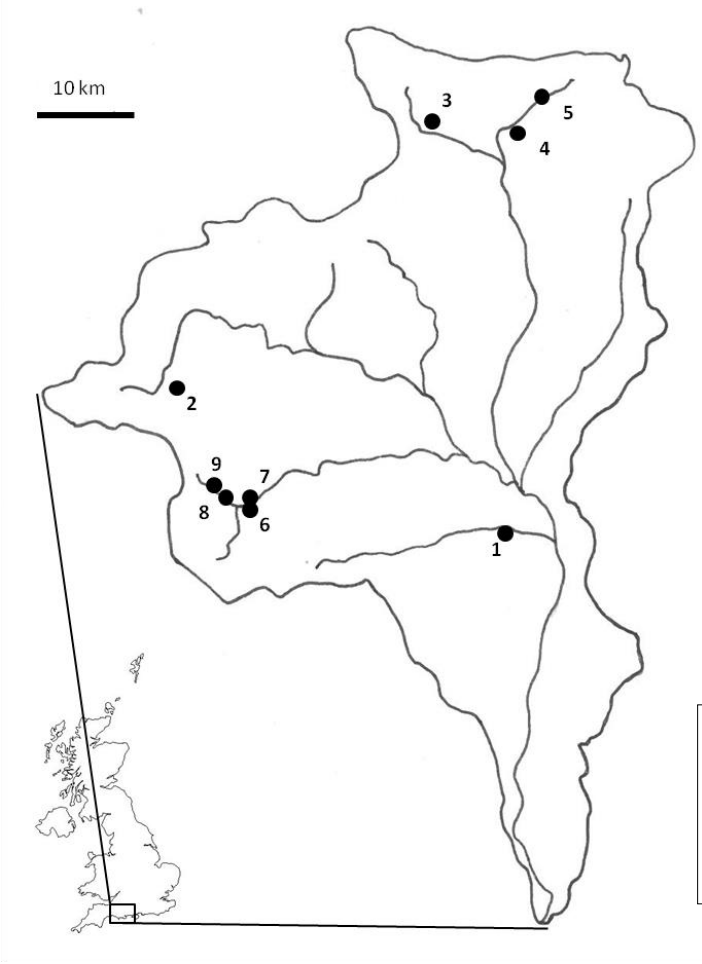

Figure 2.1: Map of the Hampshire Avon catchment showing locations of study sites. 1=Ebble, 2=Wylye, 3=Rushall-Chalk, 4=Avon, 5=Rushall-Sand, 6=Nadder, 7=Sem, 8=Priors, 9=Cools.

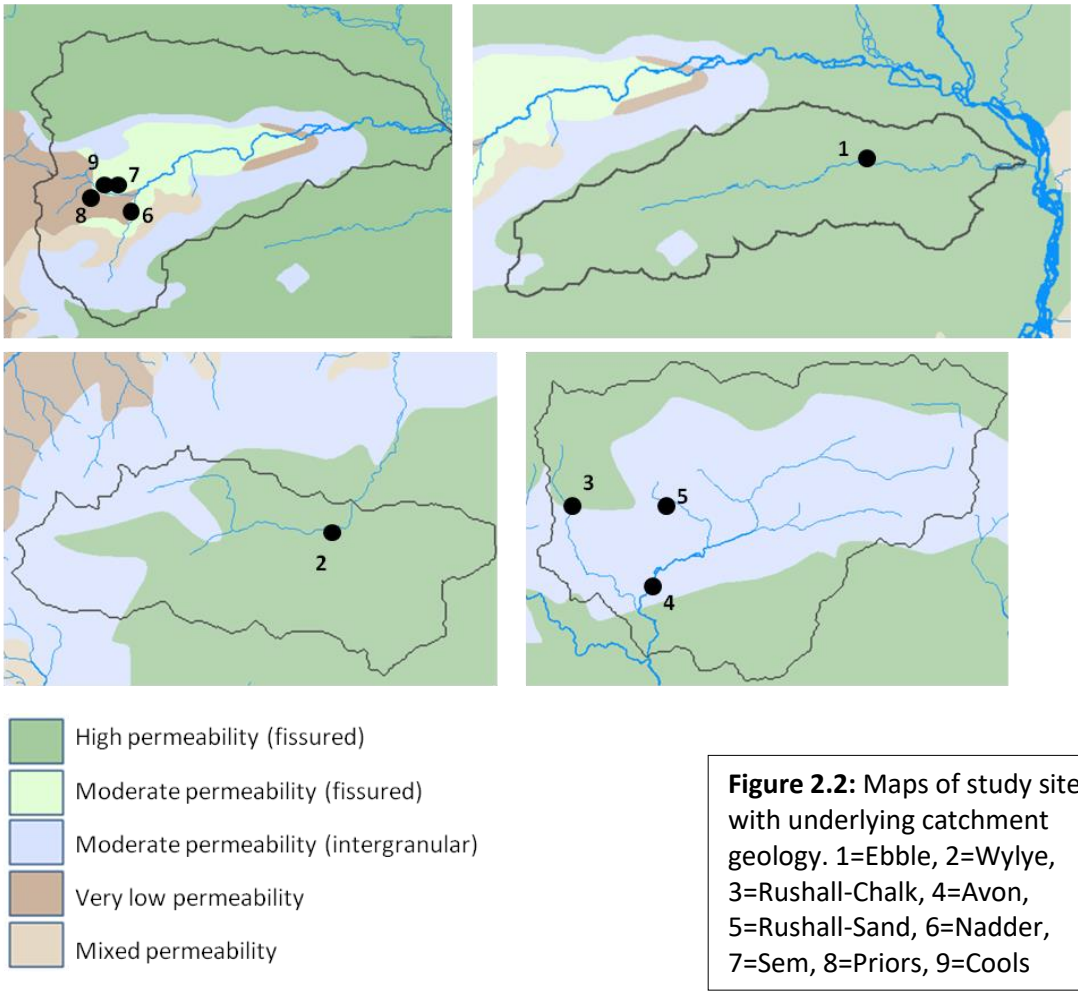

Figure 2.2: Maps of study sites with underlying catchment geology. 1=Ebble, 2=Wylye, 3=Rushall-Chalk, 4=Avon, 5=Rushall-Sand, 6=Nadder, 7=Sem, 8=Priors, 9=Cools

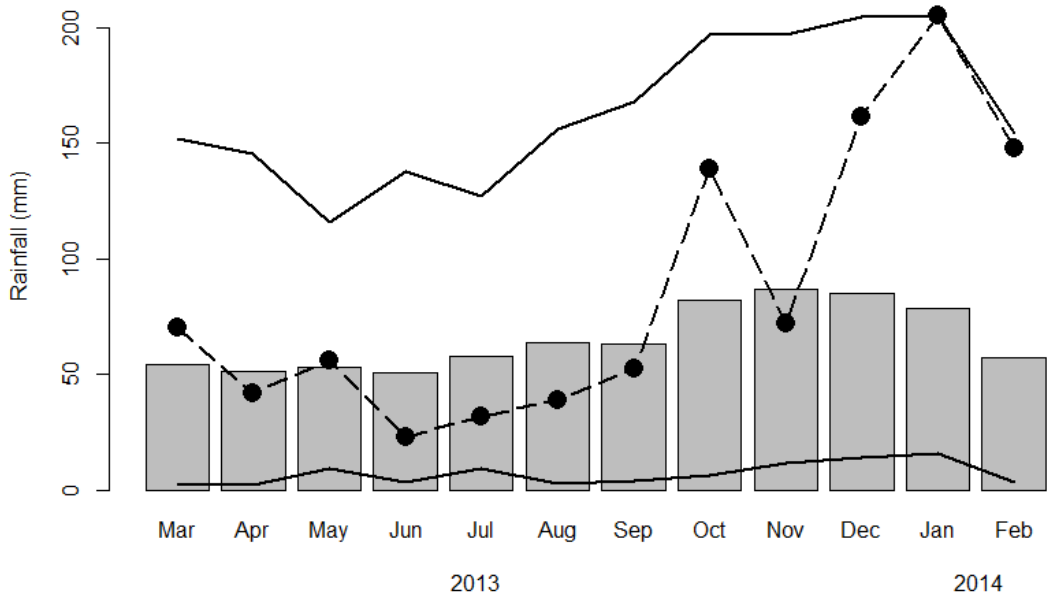

Figure 2.3: Graph showing average monthly rainfall (mm) in Southern England during the period 1910-2015 (grey bars). Minimum and maximum values over the period shown by solid lines. Rainfall during study field campaigns (2013-2014) shown by dashed line and filled points. Data downloaded from Hadley Centre long-term records (Hadley Centre 2016).

2.3 Sampling Campaigns

The sampling for the data presented in Chapter 3 was carried out during two field visits: in February 2013 and August 2013. These were to collect sediment and water samples only, which were then taken back to the laboratory to do potential measurements of CO_2 and CH_4 production, as well as other analyses. Due to flooding, it was not possible to collect samples from the Rushall-Sand site during the February visit.

The largest sampling campaign was carried out for the seasonal *in situ* study, described in chapters 4 and 5. These sampling campaigns where carried out at just the six main sites: Priors and Sem (Clay), Nadder and Avon (Sand), Wylye and Ebble (Chalk). This was carried out during 3-week periods in April 2013, July 2013, October 2013 and February 2014. During these visits measurements of benthic metabolism were carried out, alongside measurements of CO_2 and CH_4 out-gassing from the rivers to the atmosphere. Again, due to heavy flooding, some sites had to be missed. Ebble and Avon were not measured during the winter (February 2014) campaign, and only limited data was collected at the Avon in autumn (October 2013).

A final field campaign was carried out during a three week period in May 2015. This work is described in chapter 4, and the aim was to collect high-frequency measurements of CO_2 and CH_4 out-gassing from the rivers. This was carried out at the 6 main sites referred to previously.

2.3.1 Sampling Strategy

The study sites were chosen to encapsulate a geological a gradient of base flow and geology, but in addition to this, intra-site variation was considered. Chapters 3 and 4, which both measure processes in the sediment, included measurements of different types of sediment, or 'patches' within the river reaches. As discussed in Chapter 1, both between-site and intra-site variation may be hypothesized to affect carbon metabolism.

For chapter 3, which measured production of CO_2 under laboratory conditions, one chalk site (Wylye) and one sand site (Avon) were chosen to take extra samples from. These were chosen as they had high intra-reach variation, both had significant coverage of submerged vegetation, as well as areas of gravel and sand or finer sediments. The Wylye had a gravel main bed, but also large areas covered with aquatic vegetation and areas of fine sediment build up, as is typical of chalk streams. The Avon was the sand site with the most intra-site variation (compared to the Nadder), with areas of gravel as well as sand and aquatic vegetation.

Described in Chapter 4, the seasonal field campaign included consideration of intra-site variation by carrying out mapping during each field campaign. This is described in more detail in the chapter, which shows an example map (Figure 4.2) as well as the proportion of coverage of each patch type at each river, each season (Figure 4.3). Where possible, the measurements of sediment metabolism were done across the range of patch types for each river. This allowed metabolism in different patches to be compared, and when combined with the mapping data, the experiment results were scaled-up to calculate estimates of reach-scale metabolism.

2.4 Additional Data

As well as the data collected for this PhD, some data are included from other sources. This includes data collected as part of other projects in the macronutrient cycles programme, as well as data from the Demonstration Test Catchment (DTC 2010) monitoring. This includes river level, discharge and rainfall data from surface water monitoring stations.

2.5.1 Chapter 3: The potential production of CO_2 and CH_4 across geology, season and temperature.

Objectives:

- To measure sediment characteristics for the nine rivers in the study, including particle size, organic carbon content, and chlorophyll content.
- To ascertain potential rates of anoxic production of CO_2 and CH_4 in the sediments, during a period of both low and high primary production (winter and summer).
- To find how measured sediment characteristics may explain variation in CO_2 and CH_4 production.
- To determine how production of CO_2 and CH_4 are affected by temperature.
- To explain each of the above in the context of differing bed substrate properties arising from the underlying geology (chalk, sand or clay).

2.5.2 Chapter 4: How in situ carbon metabolism varies with geology, season and light.

Objectives:

- To measure rates of CO_2 and CH_4 production, along with O_2 consumption, under natural conditions.
- To map the reaches of river where the experiments are carried out in order to get an accurate measure of the areal extent of different patch types (vegetated, marginal fine sediments and the main gravel or sand riverbed) over the whole reach.
- To use the results above together with light measurements to calculate whole reach rates of ER, GPP, net ecosystem metabolism (measured as net consumption

of O_2, when both respiration and production are considered;NEM) and CH_4 production.

- To explain how these results are affected by seasonal differences, as well as across different patch types and geologies which give rise to a gradient of BFI.

2.5.3 Chapter 5: The amount of CO_2 and CH_4 out-gassed by the study rivers, and how this relates to local metabolism

Objectives:

- To measure the amount of CO_2 and CH_4 emitted from the six study rivers, with contrasting geology, during the sampling campaign of Chapter 4.
- To obtain high frequency, day and night (diel) measurements of CO_2 and CH_4 emissions from the study sites.
- To establish how emissions vary according to seasonal changes, including changes in rainfall.
- To establish what proportion of CO_2 and CH_4 out-gassed by rivers can be explained by local metabolism,by comparing these results with those in Chapter 4.

2.6 References

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3. The potential anoxic production of CO_2 and CH_4 in riverbeds across geological, seasonal and temperature gradients

3.1 Introduction

3.1.1 Background

The final stage of catabolic carbon metabolism is the release of either carbon dioxide (CO_2) or methane (CH_4) gas. CO_2 is a waste product emitted by all aerobic organisms during cellular respiration, as well being produced by some anaerobic pathways. CH_4 is emitted by a group of microbes known as methanogens. There are several groups, all within the phylum Euryarchaeota in the domain Archaea (Boone *et al.* 1993). These produce CH_4 by two main pathways: the splitting of acetate (acetoclastic methanogenesis); or hydrogenotrophic methanogenesis during which CO_2 is reduced (Schlesinger & Bernhardt 2013).

The benthic microbial community is a combination of algae, fungi, archaea and bacteria, both autotrophic and heterotrophic (Lock *et al.* 1984). Stream biofilms, also known as epilithon or periphyton, are now known to be a major feature of freshwater nutrient cycling and contribute substantially to global biogeochemical cycles (Battin *et al.* 2016). Several studies have suggested how respiration by benthic microorganisms is regulated, and relationships have been found with discharge of water (Uehlinger 2000; Acuña *et al.* 2004), nutrient availability (Howarth 1988; Stelzer *et al.* 2003) and temperature (Rees *et al.* 2005; Acuña *et al.* 2008; Bernot *et al.* 2010). Some studies have found a positive relationship with sediment organic carbon content (Hedin 1990; Acuña *et al.* 2008), though others have not (Sinsabaugh 1997; Houser *et al.* 2005). The quality of organic matter may be a more important factor, rather than the amount (Sinsabaugh 1997); as this is directly related to how amenable the organic matter is to microbial degradation.

Like respiration to CO_2, methanogenesis can also be limited by the amount of labile organic carbon present (Valentine *et al.* 1994; Denier van der Gon & Neue 1995). Additionally, controls

on methanogenesis are linked to those on other forms of respiration, and there is a positive relationship between methanogenesis and net ecosystem production (Whiting & Chanton 1993).

Given that factors such as temperature, nutrients and organic matter are known to be significant in carbon metabolism, it is therefore important to consider how these variables are affected in turn by regional, geological and land use variations. This way, small-scale data can be scaled-up using the spatial and land-use characteristics of the landscape. There have been many studies of how metabolism changes along river continua (Meyer &Edwards 1990; Wiley *et al.* 1990; Young & Huryn 1996). Rates of respiration and primary production can change along the continuum due to changes in sediment load, tree cover, flow rate and other factors (Battin *et al.* 2009). But wider surrounding land use (agricultural or urban) has been shown to be more important than in-stream or riparian differences (Bernot *et al.* 2010).

Evidently then, the wider catchment can regulate stream metabolism, due to significant lateral exchange (Cole *et al.* 2007). Underlying geology can influence lateral exchange because of the different contributions of groundwater and rainwater discussed in Chapter 1. It is also a significant predictor of factors such as macrophyte cover, light penetration and nutrient levels.For these reasons, it may be hypothesized that the geology in which a river or stream sits may be an important predictor of production of CO_2 and CH_4 by that river's benthic sediment microbial community. As discussed in Chapter 1, there are many differences in terms of sediment quality, allochthonous organic matter input, local autochthony and other characteristics which have been shown to affect respiration, and therefore CO_2 production. CH_4 production is less well studied (Stanley *et al.* 2016), but its correlation to NEP (Whiting & Chanton 1993) suggests many factors discussed here may be important. In particular, methanogenesis is strongly affected by sediment type, with rates found in fine sediments 100 times higher compared with gravel in the same river (Shelley *et al.* 2015).

All biogeochemical reactions are temperature dependent under Arrhenius' Law; however in nature enzymes are affected by a variety of environmental constraints which can complicate the response to temperature in different processes, organisms or ecosystems (Davidson & Janssens 2006). It is now known that respiration has a stronger temperature dependence than photosynthesis, in freshwaters (Yvon-Durocher *et al.* 2010a); oceans (Regaudie-de-Gioux & Duarte 2012); and terrestrial systems (Lloyd & Taylor 1994; Kirschbaum 1995). However, in terrestrial environments, any theoretical increase in respiration as a response to temperature may be hindered by lack of available organic matter produced by local primary production. Freshwaters may not have such constraints due to the potential large input of allochthonous organic material from their terrestrial catchments (Trimmer *et al.* 2012). This suggests they may be sites of positive feedbacks under global warming, with increases in respiration (i.e. emission of CO_2) that are not coupled to concurrent increases in primary production. Additionally, methanogenesis has a stronger temperature dependency than respiration (Yvon-Durocher *et al.* 2010b; Shelley *et al.* 2015). This means a greater proportion of carbon could be mineralised to Ch_4 rather than CH_2 under warming: an important consideration since CH_4 has 28 times the global warming capacity of CO_2 over 100 years (Myhre *et al.* 2013).

Similar to the overall production of CO_2 and CH_4, the experimentally measured temperature dependency of production may be hypothesized to change across a geological gradient. If certain geology or sediment types have strong constraints on methanogenesis and respiration such as nutrient limitation or redox state, these may inhibit an effective response to any increase in temperature.

In conclusion, previous studies have shown that production of CO_2 and CH_4 from freshwater sediments may be controlled by factors including light, nutrient availability and organic matter input (quantity and quality); which in turn can be affected by characteristics of

the larger riverine environment and surrounding terrestrial catchment. This suggests underlying geology is expected to influence CO_2 and CH_4, production, but there is thus far a lack of studies of this. Additionally, the difference between the temperature dependence of respiration and photosynthesis, and between respiration to CO_2 and methanogenesis, is especially significant in freshwaters and under a changing climate. Therefore, whether this is consistent or variable across a geological gradient needs to be elucidated.

3.1.2 Outline of Chapter

This chapter describes measurements of CO_2 and CH_4 production carried out under controlled laboratory conditions. These rates are combined with measurements of sediment characteristics, including particle size and organic carbon content, on the same samples in order to evaluate what may be the controlling factors on CO_2 and CH_4 production. These results will be used as part of the discussion regarding how benthic metabolism changes across a gradient of underlying geology types and between two seasons.

Additionally, because the samples were incubated at different controlled temperatures, these results are used to calculate the temperature dependencies of CO_2 and CH_4 production. Whether temperature dependence varies with geology, season or other factors can then be established; as well as comparing the temperature dependence of CO_2 production with that of CH_4 production. This is an important factor when considering riverine carbon cycling under projected warming due to future climate change.

Carrying out potential experiments under laboratory rather than *in situ* conditions allows environmental variables such as temperature and light to be controlled and kept constant, (or in the case of temperature, to change by a set amount); such that the differences in measured rates are only due to the differences in sample and river.

3.1.3 Objectives

- To measure sediment characteristics for the nine rivers in the study, including particle size, organic carbon content, and chlorophyll content.
- To ascertain potential rates of anoxic production of CO_2 and CH_4 in the sediments, during a period of both low and high primary production (winter and summer).
- To find how measured sediment characteristics may explain variation in CO_2 and CH_4 production.
- To determine how production of CO_2 and CH_4 are affected by temperature.
- To explain each of the above in the context of differing bed substrate properties arising from the underlying geology (chalk, sand or clay).

3.2 Methods

3.2.1 Study site description

These experiments were done on the nine study rivers of the Hampshire Avon, as described in Section 2.2. Three rivers are on a chalk catchment, three on sand and three are on clay. Properties of each river are summarised in Table 2.1.

As discussed in Section 2.3, the winter field campaign was carried out during a time of heavy rainfall and flooding, such that some experiments could not be undertaken at all rivers. Consequently, data for the Avon and Rushall-sand sites are incomplete. This will be explained further in the relevant sections.

3.2.2 Sample collection

Sediment samples of approximately 200 grams were taken from the top 5 cm of the nine river beds using plastic corers. They were stored in plastic Ziploc bags, squeezed and double-bagged to reduce the amount of oxygen present. The samples were stored for approximately 3 days at 5°C. Samples were homogenised and sub-samples were taken for the different experiments and sediment characteristics: these were either carried out immediately or the sub-samples were frozen at -20°C until later analysis.

The samples taken are detailed in Table 3.1. For all but two of the rivers, sediment samples (*n=5*) were taken from the main river channel, in both summer and winter (February 2013 and August 2013). For the other two of the rivers, namely the Wylye (chalk) and the Avon (sand), extra replicates were taken to incorporate three different patch types (main channel, submerged macrophyte stands of *Ranunculus* sp., and marginal sediments); resulting in 15 samples (*n= 3 patches x 5 replicates =15)* for these rivers. The purpose of these was to better characterise intra-reach variation in a heterogeneous riverbed (as described in Section 2.3.1). The extra samples are referred to throughout this chapter as 'Patch A': samples taken from

underneath vegetation; and 'Patch B': samples taken from marginal areas of fine sediment accumulation.

	Winter				Summer			
	River	Patches	Replicates		River	Patches	Replicates	
Chalk	Ebble	Main Bed	5	Chalk	Ebble	Main Bed	5	
	Wylye	Main Bed	5		Wylye	Main Bed	5	
		Vegetated (Patch A)	5			Vegetated (Patch A)	5	
		Marginal (Patch B)	5			Marginal (Patch B)	5	
	Rushall-chalk	Main Bed	5		Rushall-chalk	Main Bed	5	
Sand	Avon	Main Bed	5	Sand	Avon	Main Bed	5	
		Vegetated (Patch A)	0			Vegetated (Patch A)	5	
		Marginal (Patch B)	5			Marginal (Patch B)	5	
	Nadder	Main Bed	5		Nadder	Main Bed	5	
	Rushall-sand	Main Bed	0		Rushall-sand	Main Bed	5	
Clay	Sem	Main Bed	5	Clay	Sem	Main Bed	5	
	Priors	Main Bed	5		Priors	Main Bed	5	
	Cools	Main Bed	5		Cools	Main Bed	5	
Total			55				65	120

Table 3.1: Samples collected for analysis from the nine rivers in both seasons. Each sample was used for all experiments described in the main text, giving a total of 120 measurements of each analysis. Where 0, adverse weather conditions prevented sample collection.

3.2.3 Laboratory analysis

Potential CO_2 and CH_4 production as a function of temperature:

For these measurements, each of the samples described in Table 3.1 (*n*=120) were divided into three, in order to measure each at 3 temperatures. To measure the potential for anerobic CO_2 and CH_4 production, sediment (~3g) was measured into replicate (*n=3*) 12 mL gas-tight vials (Exetainers, Labco, UK). These were then transferred to an anoxic glove-box (Belle Instruments, UK), where de-gassed river water (1 mL) was added before they were sealed. Following sealing, the headspace concentration of CO_2 and CH_4 was measured using a gas chromatograph fitted with a flame ionising detector (GC-FID), (Agilent Technologies, UK; full method described in (Sanders *et al.* 2007)). The GC separated CO_2 and CH_4 in a column packed with Porapak, with a hydrogen and air mixture as the carrier gas. The CO_2 was then converted to CH_4 via a nickel catalyst at 385˚C. The CH_4 was oxidised and detected by the FID, giving a peak area.Concentrations were calculated from peak areas by calibration with known

standards. Precision was measured as a coefficient of variation of better than 2%, for both CO_2 and CH_4 measurements. Repeat measurements of CO_2 and CH_4 were carried out at timed intervals approximately once every 24-48 hours for 10 days. In between measurements, the vials were incubated at three temperatures: 5, 10 and 22°C.

Due to the sample preparation techniques described above, these experiments were carried out under anoxic conditions. This was because these conditions were more similar to the *in situ* conditions than a fully oxic environment would be; and also to ensure potential production of methane by strictly anoxic methanogens was maximized. However, the limitations of this technique are that CO_2 production would likelybe lower than that found *in situ*, and any methanotrophic bacteria present would be inhibited. As such, results from these experiments are not intended to be used as an exact prediction of rates *in situ*. Instead, they are used to compare differences between sites, geologies and temperature treatments; and how the sediment characteristics measured can predict potential production of CO_2 and CH_4.

From the concentrations measured by gas chromatography, the total amount of CO_2 and CH_4 in the vial (gas and water) was calculated using Henry's law and solubility coefficients for Co_2(Weiss 1974) and CH_4(Yamamoto *et al.* 1976). Rates of CO_2 and CH_4 production were calculated using linear regression over the first 100-130 hours of incubation (linear phase) and presented as μg C g dry wt^{-1} hr^{-1}.

Parallel samples were used to measure sediment characteristics, namely microbial activity, chlorophyll content, particle size, organic carbon and nitrogen content and carbon stable isotope ratios. All measurements were carried out on each of the samples described in Table 3.1 (*n = 120*).

Microbial activity:

Microbial activity was measured by adding fluorescein diacetate (FDA) to sediment subsamples in phosphate buffer. FDA is hydrolysed by many enzymes involved in biomass decomposition including proteases and estarases, and so be used as an estimate of the size of the microbial biomass pool (Sánchez-Monedero *et al.* 2008). This was carried out according to the method described in Schnurer & Rosswall (1982). A control sample, prepared the same as the test samples but without adding the FDA, was also prepared for each sample.

The samples were incubated for approximately five minutes then the reaction was terminated by adding acetone. The absorbance of the supernatant at 490 nm was then measured on a spectrophotometer and compared with the control sediments. Results were corrected by subtracting the absorbance of the control sample (per gram dry weight) from the absorbance of the treatment sample. Then the rate of FDA hydrolysis of each sample was calculated according to the following equation:

$$\text{Rate of FDA hydrolysis } (\mu\text{mol g}^{-1} \text{ dry sediment min}^{-1}) = \frac{\text{ABS}_{490}}{81.3} \text{ x } \frac{\text{V}_a}{\text{M}_d} \text{ x } \frac{1}{\text{time}}$$

Where:

ABS_{490} = absorbance of sample at 490nm due to the presence of fluorescein (i.e. measured absorbance of sample – absorbance of control)

81.3 is the molar absorptivity coefficient for FDA (mM cm^{-1}, determined previously)

V_a = volume of the assay (mL buffer + mL acetone + mL FDA)

M_d = mass of dry sediment (g)

Time = Amount of time between the addition of FDA and termination of the reaction by acetone (min).

Chlorophyll content:

This was carried out to obtain a proxy measure of the lability of carbon present in the sediments.

To measure chlorophyll content, acetone was added to dried subsamples, they were shaken and incubated overnight then the absorbance was measured on a spectrophotometer at 664, 665 and 750 nm. Samples were then acidified by adding 10 µl 1M hydrochloric acid,

and the absorbance was measured again. (Lorenzen 1967; Dalsgaard 2000). Results were used to find the chlorophyll-a concentration of the samples, in μg g^{-1} dry weight using the following equation:

$$[\text{Chlorophyll a}](\mu\text{g g}^{-1}\text{ dry sed}) = \frac{11 * 2.43 * \left[\left(\text{ABS}_{664_\text{B}} - \text{ABS}_{750\_\text{B}}\right) - \left(\text{ABS}_{665\_\text{A}} - \text{ABS}_{750\_\text{A}}\right)\right] * \text{V}_\text{e}}{\text{M}_\text{d}}$$

Where:
11 and 2.43 are constants
ABS = absorbance and 664, 665 and 750 refer to the wavelength (nm) of the absorbance measurement
B = before acidification
A = after acidification
Ve = volume of extract (mL of acetone added)
Md = mass of dry sediment (g)

Organic carbon and nitrogen content, and $\delta^{13}C$:

The total organic carbon and nitrogen present in the sediment was measured in each of the samples, to determine the amount present (both labile and refractory) in the sediment. Additionally, the $\delta^{13}C$ of the organic carbon was measured. This was used to discern any differences in isotope signals between seasons or sites, which may suggest differences in the origin of the organic matter.

Dried subsamples (approx. 3 g) were sieved to 2 mm then powdered using a pestle and mortar and weighed. They were then acidified to remove inorganic carbon by treatment with 1M hydrochloric acid (Hedges & Stern 1984). After approximately 48 hours the acid was removed using a pipette and they were dried. Smaller subsamples were transferred into ultra-clean tin caps and weighed using a microbalance (Mettler-Toledo GmbH, Laboratory & Weighing Technologies, Switzerland). A continuous flow stable isotope ratio mass spectrometer (IRMS) with elemental analyser (Sercon Integra2 Stable Isotope Analyser, precision as coefficient of variation was calculated to be better than 5%) was used to combust the samples and determine the percentage carbon and nitrogen content, as well as the $\delta^{13}C$ of

the carbon. The sample is ionised by going through a combustion column (700˚C) and an oxidation column (1000˚C) in turn with a carrier gas (helium). The ionised sample is separated using electromagnetic deflection and the isotopes then go through a detector.Organic carbon and nitrogen content of the original, un-acidified sample was calculated using the weights of the samples before and after acidification.

Particle size:

Particle size analysis was carried out in order to quantify both the average size of sediment particles, as well as measure what proportion of each sediment was made up of clay and silt.

Large (>100 g) subsamples were used to measure particle size. They were first dried, and any visible organic matter (leaves, twigs) were removed by hand. They were then manually sieved through laboratory test sieves (Endecotts Ltd, London) of various sizes (37.5, 19.0, 9.5, 4.75, 2.0 and 1.0 mm). The <1 mm fraction was treated with 30% hydrogen peroxide to remove organic matter, and then measured by laser-diffraction (LS100 Beckman Coulter Counter; Beckman Coulter, Fullerton, CA, USA). The results were expressed in percentage by weight in each size interval, and cumulative percentage. Percentage of each sediment type (clay (<2µm), silt (2-63 µm) sand (63-2000 µm), or gravel (>2000µm)) was determined, as well as the median particle size (d50).

3.2.4 Data analysis

Sediment characteristics:

In order to explore important characteristics of sediment across different rivers and geology, the results of the sediment characteristic analysis (particle size, chlorophyll content, organic carbon and nitrogen content and $\delta^{13}C$) were used to construct a principal component analysis (PCA) in R (R Core Team 2015) and draw a bi-plot of principal components 1 and 2

using ggplot (Wickham 2009). Analyses were carried out on both summer and winter data. Differences between winter and summer were also investigated using *t*-tests.

Potential anoxic CO_2 and CH_4 production

The rates of production were calculated in μC g dry wt^{-1} hr^{-1} for CO_2 and ng C g dry wt^{-1} hr^{-1} for CH_4, then expressed as a natural logarithm. Incubation temperatures were converted to standardised temperature, $1/(k/T)$, where T is the temperature in Kelvin and k is the Boltzmann constant, 8.6173324 x 10^{-5}. They were centred around 10°C such that when rates were plotted against temperature, the intercept corresponds to the predicted rate at 10°C. This gave one rate for each sample described in Table 3.1. This value was then used to compare the rates between contrasting bed substrates arising from different rivers, geologies and seasons.An average rate at 10˚C was used as this corresponded closely to the temperature*in situ*. Using one rate per sample rather than all three simplified the analysis and preserved degrees of freedom; allowing the comparisons between variables to be investigated without extra complicating factors. The full range of results, using all the temperature experiments, were included in the later analysis of temperature control on potential production of CO_2 and CH_4.

Generalised linear and linear mixed effects models were designed using R and the *nlme* package (R Core Team 2015; Pinheiro et al. 2015) to investigate the effect of geology and season on potential anaerobic CO_2 and CH_4 production. Starting with null models comparing just the intercept with production, models were built up adding variables and in some cases, random effects (e.g. river); and Akaike information criterion (AIC) values were compared to help decide which models were best at describing the data.

Natural logged rates and the standardised temperature calculated as decribed above were used to calculate the slope.The slope is the apparent activation energy (eV) of CH_2 and CH_4 production for each sample. The activation energy represents how responsive to a change in temperature the sediment is: i.e. the temperature dependency. The temperature dependency

was then examined to find how this changed with season and geology using mixed linear effects models. The difference between the temperature dependency of CO_2 and CH_4 was found using an ANOVA model. These analyses were repeated using just the patch samples collected from the Avon and the Wylye, in order to find how much intra-reach variation exists, and how this compared to variation between sites.

Lastly, the ratio between CO_2 and CH_4 production at each temperature was calculated, to investigate how the proportion of total carbon mineralised to CH_4 rather than CO_2 changes with temperature.

3.3.1 Sediment characteristics

The results of the analysis of sediment characteristics are summarised in Table 3.2. The results in summer are also presented in Figure 3.1. All characteristics were measured during both summer and winter sampling campaigns, except Avon Patch 2 and Rushall-sand, which were not sampled in the winter campaign due to adverse weather conditions. Seasonal differences were tested using paired *t*-tests. Both microbial activity and chlorophyll were higher in summer than winter. Chlorophyll was on average 0.80 µg g dw^{-1} higher in summer (T_{10}=2.46, *P*=0.03) and microbial activity was 26.7 µmol g^{-1} min^{-1} higher (T_{10}=12.01, p<0.001). However, no seasonal differences in particle size, organic carbon, nitrogen or $\delta^{13}C$ were found, when considering the whole dataset.

The principal component analyses were carried out using the main bed samples, as well as the patch samples where available (Wylye and Avon, described in Section 3.2.2), for a total of *n = 65* in summer and *n = 55* in winter. For summer, principal components 1 and 2 (PC1 and PC2) explained the variation in sediment characteristics well, describing a total of 85.7%; though for winter this was slightly less at 70.9%. During both summer and winter, the most significant characteristics explaining PC1 were organic carbon and nitrogen content, with chlorophyll and particle size the most significant variables in PC2 (Table 3.3).

Components 1 and 2 were used to draw a bi-plot for each season (Figure 3.2), to illustrate patterns across geology. Whilst it is clear in the summer bi-plot with the higher percentage of variation explained, both bi-plots show there are some common characteristics across each geology. In summer, the clay rivers are clusters at the bottom of the bi-plot, with lower PC2 values than sand or chalk: mostly due to low chlorophyll content. In winter, clays still fall lower than chalks but sand sites also have low PC2 values. Clay site samples also have more negative PC1 values, with higher organic carbon and nitrogen content. The chalk sites tend to have the

largest ovals, indicating higher levels of intra-site variation as well as positive PC2 values due to their high chlorophyll contents. Some chalk samples have PC1 values close to those of clay sites, indicating areas of fine sediment deposition.

Despite these general patterns between geology type, however, there is still considerable overlap between rivers of different geology. This suggests underlying geology alone is not enough to fully predict the characteristics of a river sediment.

Table 3.2: Summary of sediment characteristics for each river, summer and winter sampling campaigns, *n*=5, standard errors in brackets. NA=Not Available: due to adverse weather conditions, it was not possible to collect samples from Avon Patch A or from Rushall-sand during the winter campaign.

River Name	Site Code	Geology	Season	Chlorophyll ($\mu g\ g\ dw^{-1}$)	Organic Carbon (% dw)	Nitrogen (% dw)	$\delta^{13}C$	Microbial Activity (FDA, $\mu mol\ g\ dw^{-1}\ min^{-1}$)	Particle Size (Median diameter, µm)
Ebble	CE1	Chalk	Winter	1.49 (±0.30)	2.71 (±1.38)	0.09 (±0.05)	-26.53 (±1.16)	8.03 (±0.95)	4950.00 (±1349.54)
			Summer	1.49 (±0.43)	0.83 (±0.11)	0.07 (±0.02)	-27.61 (±0.56)	24.90 (±2.68)	1600.00 (±244.95)
Wylye	CW2	Chalk	Winter	0.83 (±0.17)	2.73 (±2.20)	0.10 (±0.08)	-26.77 (±1.08)	4.24 (±0.32)	8200.00 (±3242.68)
		(main bed)	Summer	2.26 (±0.44)	0.50 (±0.06)	0.05 (±0.00)	-27.09 (±0.62)	31.92 (±5.53)	3875.00 (±1875.00)
Wylye (Patch A)	CW2	Chalk	Winter	0.23 (±0.03)	15.66 (±0.61)	0.69 (±0.06)	-31.74 (±0.30)	14.91 (±3.14)	28.76 (±5.05)
		(under vegetation)	Summer	3.96 (±1.29)	3.54 (±0.73)	0.31 (±0.06)	-29.05 (±0.98)	40.18 (±6.45)	3375.00 (±793.86)
Wylye	CW2	Chalk	Winter	0.26 (±0.07)	5.22 (±0.89)	0.18 (±0.03)	-29.12 (±0.34)	10.41 (±1.88)	148.04 (±23.92)
(Patch B)		(marginal fines)	Summer	0.65 (±0.26)	3.54 (±0.64)	0.34 (±0.06)	-28.33 (±0.49)	42.28 (±5.61)	210.59 (±197.36)
Rushall-Chalk	CA3	Chalk	Winter	0.70 (±0.22)	0.50 (±0.13)	0.05 (±0.01)	-30.69 (±1.49)	2.51 (±0.67)	273.98 (±18.15)
			Summer	2.03 (±0.67)	4.24 (±1.77)	0.33 (±0.14)	-27.75 (±1.04)	39.74 (±6.74)	166.56 (±68.47)
Avon	GA2	Greensand	Winter	0.67 (±0.15)	2.18 (±0.93)	0.08 (±0.01)	-31.78 (±1.79)	0.97 (±0.27)	87.96 (±39.46)
		(main bed)	Summer	0.92 (±0.06)	0.42 (±0.07)	0.05 (±0.02)	-26.68 (±0.15)	27.90 (±2.81)	297.92 (±70.68)
Avon	GA2	Greensand	Winter	NA	NA	NA	NA	NA	NA
(Patch A)		(under vegetation)	Summer	0.53(±0.04)	0.52 (±0.18)	0.05 (±0.02)	-26.81 (±0.07)	30.94 (±2.07)	529.93 (±153.62)
Avon	GA2	Greensand	Winter	0.11(±0.04)	0.73 (±0.18)	0.08 (±0.01)	-30.57 (±1.14)	4.59 (±0.47)	195.60 (±11.92)
(Patch B)		(marginal fines)	Summer	0.71(±0.28)	0.48 (±0.17)	0.04 (±0.02)	-26.65 (±0.26)	28.50 (±3.09)	207.10 (±13.72)
Rushall-Sand	GA3	Greensand	Winter	NA	NA	NA	NA	NA	NA
			Summer	1.17(±0.38)	0.48 (±0.08)	0.05 (±0.01)	-26.30 (±0.26)	27.68 (±1.24)	3650.00 (±673.61)
Nadder	GN1	Greensand	Winter	0.34(±0.12)	0.54 (±0.19)	0.04 (±0.01)	-25.68 (±0.54)	4.18 (±0.29)	904.42 (±447.41)
			Summer	1.02(±0.62)	0.59 (±0.19)	0.03 (±0.01)	-25.89 (±0.33)	24.52 (±1.97)	418.81 (±103.78)
Sem	AS1	Clay	Winter	0.10(±0.06)	1.85 (±0.71)	0.12 (±0.03)	-25.26 (±0.90)	8.79 (±3.81)	92.56 (±46.99)
			Summer	0.36(±0.07)	2.33 (±0.60)	0.16 (±0.03)	-26.95 (±0.34)	25.56 (±2.57)	332.82 (±89.72)
Priors	AS2	Clay	Winter	0.24(±0.16)	6.59 (±0.70)	0.32 (±0.05)	-27.44 (±0.50)	2.62 (±1.51)	2226.58 (±1827.79)
			Summer	0.54(±0.15)	6.60 (±0.46)	0.49 (±0.03)	-28.81 (±0.09)	42.02 (±7.33)	332.82 (±89.72)
Cools	AS3	Clay	Winter	0.23(±0.07)	4.03 (±0.68)	0.28 (±0.05)	-28.97 (±0.41)	6.17 (±0.34)	122.74 (±55.20)
			Summer	0.26(±0.06)	2.96 (±0.65)	0.19 (±0.03)	-27.65 (±0.62)	30.78 (±3.79)	155.15 (±63.01)

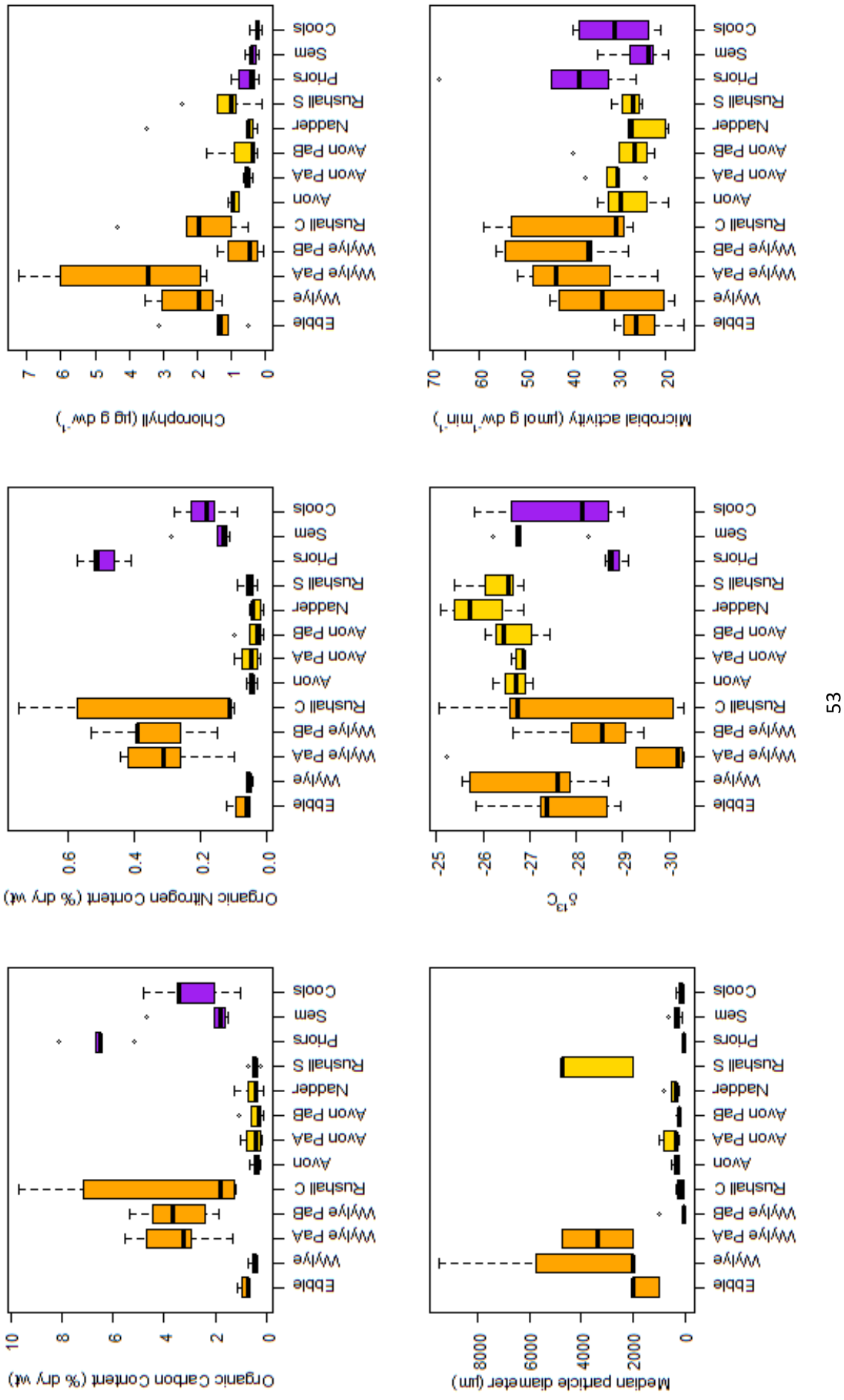

Figure 3.1: Box-plots of sediment characteristics for each river, summer sampling campaigns (*n=5*). Colours denote geology type: Orange = Chalk; Yellow = Sand; Purple = Clay.

Winter	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5
Chlorophyll	0.248	**0.664**	**0.536**	0.457	-0.007
Organic carbon	**-0.563**	0.359	-0.249	0.087	**0.696**
Nitrogen	**-0.584**	0.288	-0.214	0.138	**-0.715**
d13c	0.401	0.005	**-0.698**	**0.593**	-0.002
Particle size	0.345	**0.589**	-0.342	**-0.642**	-0.067
Cumulative proportion	**0.481**	**0.709**	**0.878**	**0.989**	**1.000**

Summer	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5
Chlorophyll	-0.219	**0.668**	**0.699**	-0.129	-0.002
Organic carbon	**-0.581**	-0.151	-0.114	-0.403	**-0.681**
Nitrogen	**-0.590**	-0.116	-0.127	-0.302	**0.729**
d13c	**0.511**	-0.124	0.124	**-0.839**	0.067
Particle size	0.075	**0.708**	**-0.683**	-0.161	-0.013
Cumulative proportion	**0.556**	**0.857**	**0.950**	**0.997**	**1.000**

Table 3.3: PCA loadings of sediment characteristics, for winter samples (top) and summer samples (bottom), for all rivers. Most significant variables (i.e. highest scores) for each component highlighted in bold. Cumulative proportion indicates how much variation is described by adding each component. Component 1 (PC1) is the component that describes the most variation, i.e. the most significant variables in PC1 are the most significant overall.

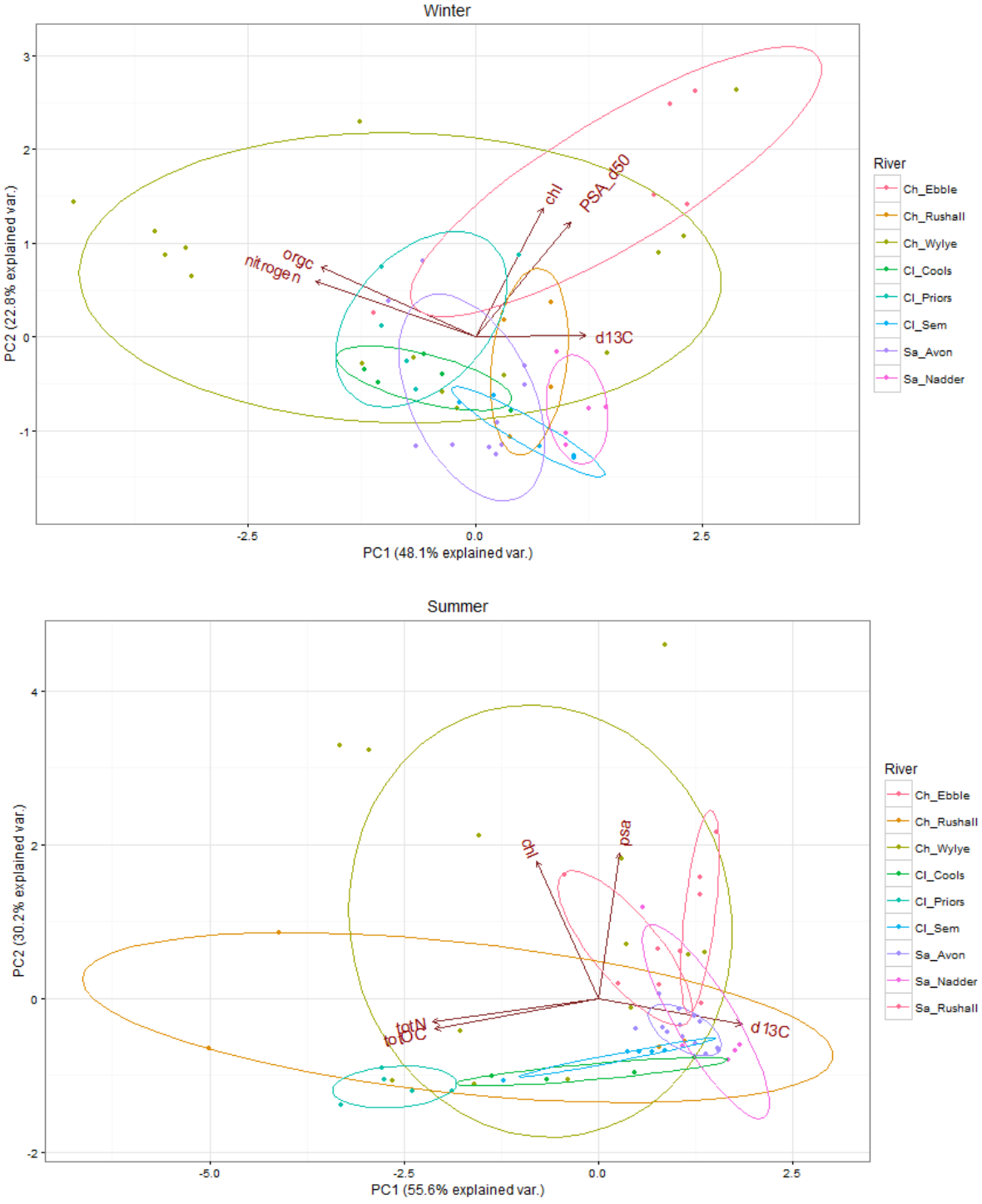

Figure 3.2: Bi-plots of principle components 1 and 2, for winter (top) and summer (bottom), showing variation in PC1 and PC2 for each river. (Prefix to each river name denotes geology type: Ch is chalk, Cl is clay, Sa is sand.)

3.3.2 Potential anoxic production of CO_2 and CH_4

Average production of CO_2 and CH_4 at each temperature measured are shown in Table 7.1 and 7.2 (Appendix). These results are summarised in Figure 3.3. Samples collected from the Wylye (chalk) Patch A (under submerged vegetation) consistently show the highest rates of both CO_2 and CH_4 production. These were highest in winter, rates of 1.27 ±0.28 µgC g dw^{-1} hr^{-1} of CO_2 and 1889 ±354 ngC g dw^{-1} hr^{-1} of CH_4. In general, though, production in summer was higher than in winter. The lowest rates were seen in some of the sand sites, with the Avon main bed only producing 0.01 ±0.00 µgC g dw^{-1} hr^{-1} of CO_2 and 0.01 ±0.00 ngC g dw^{-1} hr^{-1} of CH_4 in winter.

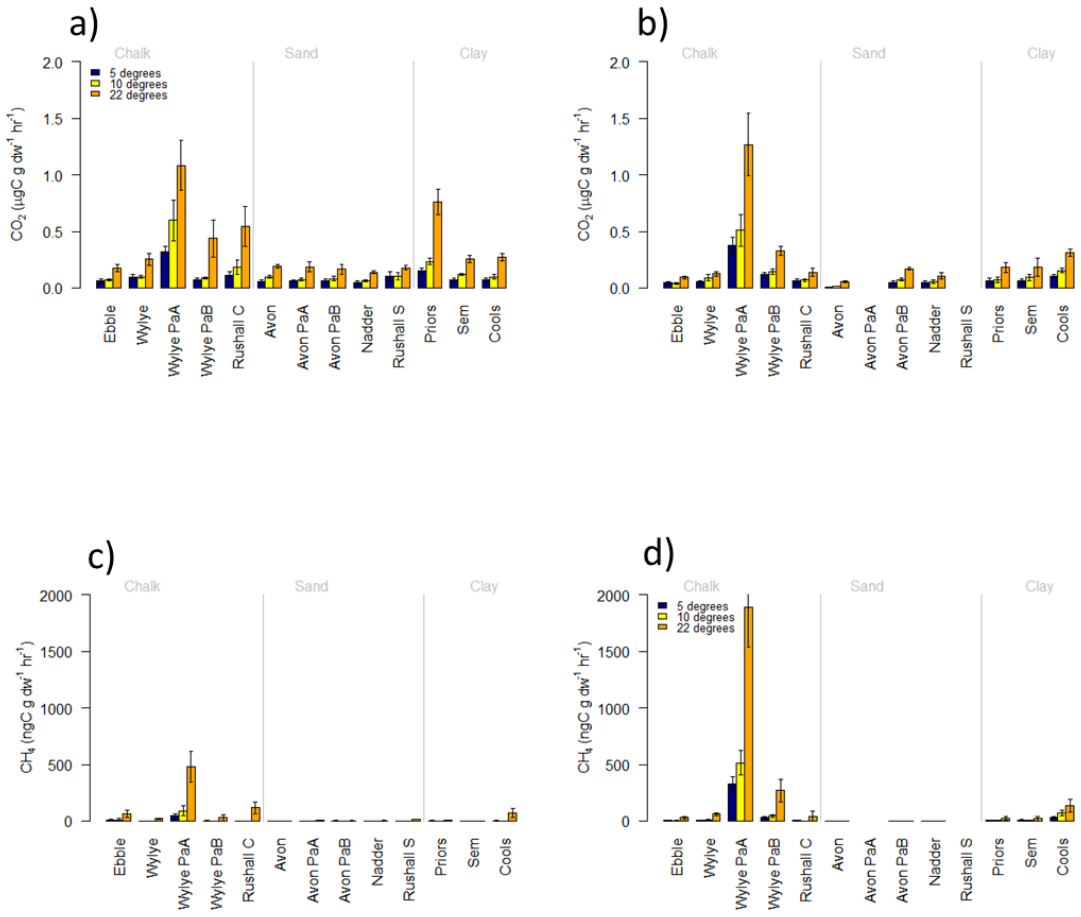

Figure 3.3: Rates of carbon dioxide (a and b) and methane (c and d) potential production, measured over 3 temperatures in summer (a and c) and winter (b and d).

Average production ratesat 10°C, calculated using all the temperature measurements described above, are shown in Table 3.3, as well as Figure 3.4. All samples produced CO_2, but CH_4 production was much more heterogeneous, with a small number of samples producing none (limit of detection 0.01 ngC g dw^{-1} hr^{-1}), but some having very high rates of methanogenesis; in some cases, as high as CO_2 production.

		Carbon Dioxide µgC g dw^{-1} hr^{-1}				Methane ngC g dw^{-1} hr^{-1}				Percentage of C emitted as CH_4			
	River	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*
Chalk	Ebble	0.08	*0.02*	0.05	*0.01*	13.03	*10.50*	4.72	*3.11*	8.21	*5.12*	6.49	*2.71*
	Wylye	0.12	*0.01*	0.07	*0.01*	1.62	*0.58*	9.63	*3.16*	1.28	*0.35*	10.64	*3.12*
	Wylye Patch A	0.49	*0.08*	0.53	*0.12*	87.68	*33.53*	541.53	*105.15*	13.36	*4.19*	50.50	*4.16*
	Wylye Patch B	0.11	*0.01*	0.15	*0.02*	1.58	*1.42*	51.27	*19.09*	1.22	*1.08*	21.36	*7.81*
	Rushall-chalk	0.18	*0.06*	0.08	*0.02*	2.74	*0.80*	2.42	*2.24*	1.67	*0.60*	1.74	*1.53*
Sand	Avon	0.09	*0.01*	0.02	*0.00*	0.13	*0.03*	0.01	*0.00*	0.15	*0.02*	1.04	*0.96*
	Avon Patch A	0.08	*0.01*	NA	*NA*	0.40	*0.31*	NA	*NA*	0.35	*0.22*	NA	*NA*
	Avon Patch B	0.08	*0.02*	0.07	*0.01*	1.11	*0.92*	0.13	*0.06*	1.36	*1.11*	0.16	*0.05*
	Nadder	0.06	*0.01*	0.06	*0.02*	0.31	*0.17*	0.35	*0.28*	0.47	*0.17*	0.91	*0.60*
	Rushall-sand	0.11	*0.03*	NA	*NA*	2.04	*0.51*	NA	*NA*	1.84	*0.29*	NA	*NA*
Clay	Sem	0.11	*0.01*	0.09	*0.03*	0.87	*0.35*	9.59	*9.24*	0.70	*0.24*	4.26	*3.40*
	Priors	0.24	*0.03*	0.08	*0.02*	2.24	*0.86*	6.56	*3.42*	0.96	*0.39*	5.87	*2.65*
	Cools	0.10	*0.02*	0.15	*0.01*	5.06	*2.37*	53.56	*17.88*	3.83	*1.42*	23.25	*6.77*

Table 3.4: Average production rates of carbon dioxide and methane at 10°C, and the proportion (%) of total carbon mineralised as methane (*n=5*, standard errors in italics).

River	Model structure	AIC	Response variable	Random variable	Explanatory variable	df	F	p
All (main bed)	geology+season	164.2	logged-CO2	season|river	geology	2,6	6.28	0.034
	linear mixed effect				season	1,75	7.83	0.007
All (main bed)	geology*season	342.0	logged-CH4	River	geology	2,6	3.44	0.101
	linear mixed effect				season	1,70	0.13	0.719
					geology*season	2,70	3.55	0.034
Wylye (chalk)	season*patch	120.4	logged-CH4	NA	season	1,24	14.05	0.001
	generalised linear				patch	2,24	18.84	<0.0001
					season*patch	2,24	1.11	0.346
Wylye (chalk)	patch	40.0	logged-CO2	NA	patch	2,27	52.14	<0.0001
	generalised linear							
Avon (sand)	season+patch	50.5	logged-CO2	NA	season	1,21	15.83	0.001
	generalised linear				patch	2,21	4.40	0.025
Avon (sand)	season+patch	83.8	logged-CH4	NA	season	1,20	8.42	0.009
					patch	2,20	2.73	0.090

Table 3.5: Summary of model results, comparing CO_2 and CH_4 production across seasonal, geology and patch differences.

The models used to investigate seasonal, geology and patch differences between CO_2 and CH_4production are summarised in Table 3.5. Comparing the main bed samples from each river (*n=5* for each river, no patch samples); season is a significant factor in CO_2 production ($F_{2,6}$=6.277, p=0.0338). In winter, around 0.053 µg C g $dw^{-1}hr^{-1}$ less CO_2 was produced in winter compared to summer. This was consistent across geology type. However,analysis of CH_4 production did not show any differences between summer and winter, although the interaction between season and geology was significant ($F_{2,70}$=3.552, p=0.0339), as the chalk and clay sites produced slightly more in winter, and sand slightly less.

The effect of geology on CO_2 and CH_4 production was also tested, to find whether there were general trends between each group of river samples: chalk, clay and sand. Geology was a significant factor in CO_2 production ($F_{2,6}$=6.277, *p*=0.0338), with the highest rates occurring in the clay sites, at 0.148 µg C g $dw^{-1}hr^{-1}$(in summer). Chalk was similar to clay with 0.11 µg C g $dw^{-1}hr^{-1}$, but sand considerably lower at 0.0668 µg C g $dw^{-1}hr^{-1}$. When CH_4results was tested, geology was not found to be a significant predictor of production.

For the two sites that were the focus of intra-site variation (Wylye and Avon); generalised linear models were used to calculate what difference, if any, there was between the main river bed sediment and the two patches. Patch A was sediment that had built up underneath macrophytes, and patch B was fine, marginal sediment. For the Wylye (a chalk site), patch type was a highly significant predictor of both CO_2 ($F_{2,27}$=52.144, *p*<0.0001), and CH_4 ($F_{2,24}$=18.840, *p*<0.0001) production. Patch A had the highest average production of both CO_2 and CH_4, at 0.467 µg C g $dw^{-1}hr^{-1}$ and 54.402 ng C g $dw^{-1}hr^{-1}$ in summer, respectively. Patch B was more similar to the main bed, with 0.121 µg C g $dw^{-1}hr^{-1}$ CO_2 and 2.848 ng C g $dw^{-1}hr^{-1}CH_4$ produced in patch B, compared to 0.0895 µg C g $dw^{-1}hr^{-1}$ CO_2 and 1.247 ng C g $dw^{-1}hr^{-1}$ CH_4 in the main bed. However, when the same analysis was carried out on the Avon (a sand site), no differences were found between the main bed and the patch samples.

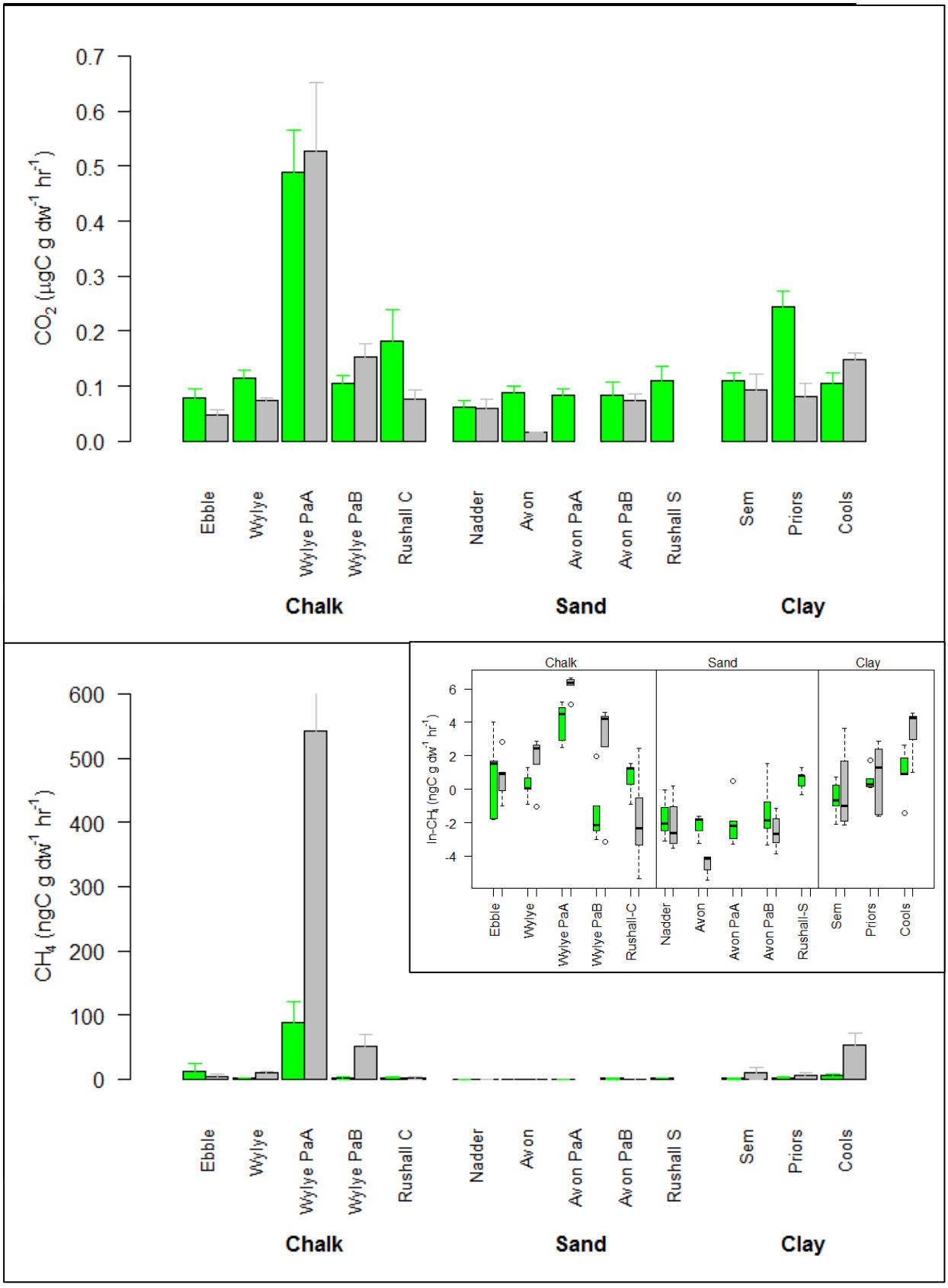

Figure 3.4: Potential anaerobic production of CO_2 (top) and CH_4 (bottom) during summer and winter, with natural logged CH_4 in bottom inset. Bars are mean values (*n=5*), error bars show 1 standard error. Missing results for Rushall-Sand and Avon patch A in winter are due to adverse weather conditions preventing sample collection.

The potential production of CO2and CH4 were modelled against each explanatory characteristic and against PC1 and PC2 from the principle component analysis. The relationships are shown in Table 3.6. This analysis included the patch samples for the Wylyeand Avon as well as the main bed samples for all the rivers. PC1 could explain a large amount of the variation in CO2 in both summer and winter, with r^2 values of 0.506 ($p < 0.001$) and 0.361 ($p < 0.001$) respectively. However, CH_4 production was less dependent on PC1, with summer and winter r^2 values of 0.182 ($p < 0.001$) and 0.195 ($p = 0.001$) respectively. Of the individual explanatory characteristics, organic carbon and nitrogen content were most closely correlated with potential production of CO_2 and CH_4 overall. These were the main components of PCA1. Both organic carbon and nitrogen content were significantly correlated with production of CO_2 and CO_2 in both summer and winter. The relationship between production of CO_2 and CH_4 and organic carbon content is shown in Figure 3.5. However, the other sediment characteristics (Chlorophyll-a, $\delta^{13}C$ and particle size) were less related to either CO_2 or CH_4 production. Although relationships are seen with the summer samples, there are no significant relationships seen with the winter sediment samples.

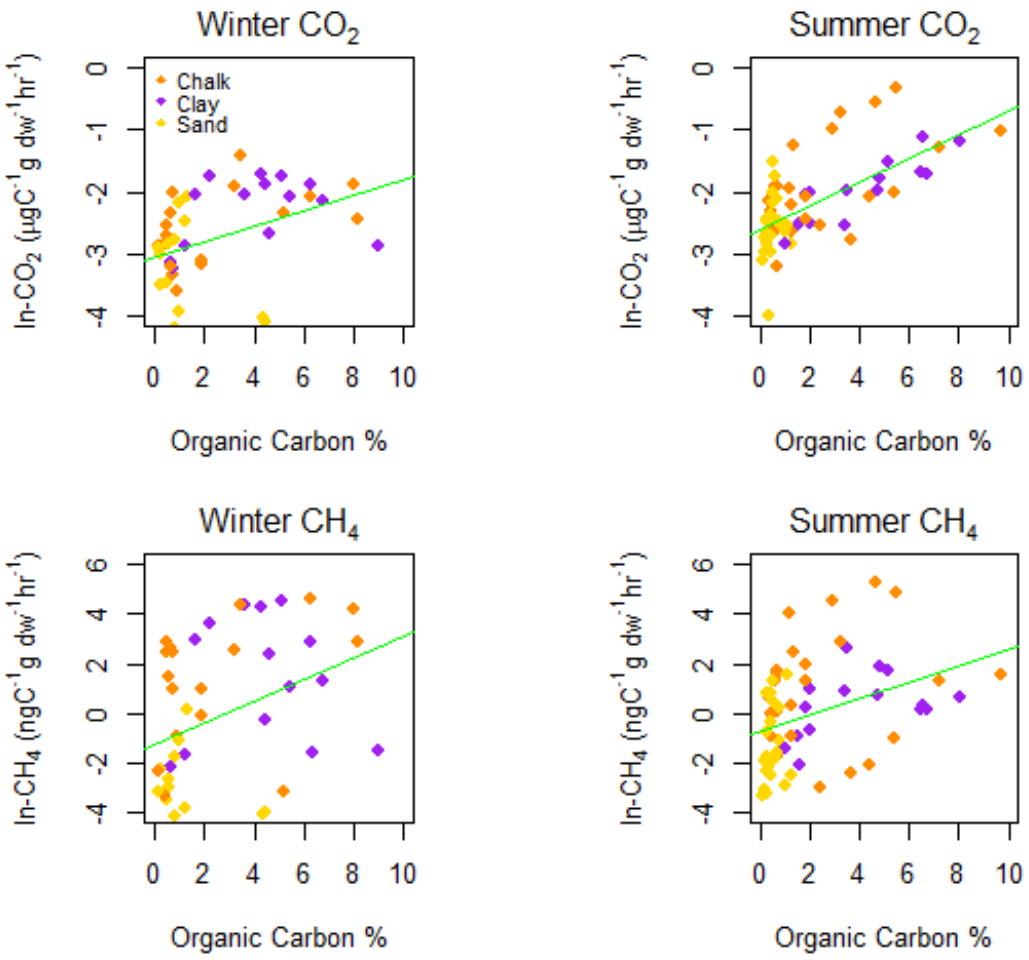

Figure 3.5: Relationship between CO_2 and CH_4 production organic carbon content, by percentage of dry weight.

Season	Gas	Explanatory Characteristic										Principle Component			
		Organic Carbon Content (% dry wt)		Organic Nitrogen Content (% dry wt)		Chlorophyll-a (μg g $dw^{-1)}$		$\delta^{13}C$		Median Particle Diameter (μm)		PC1		PC2	
		r^2	*p value*	r^2	*p value*	r^2	*p value*	r^2	*p value*	r^2	*p value*	r^2	*p value*	r^2	*p value*
Summer	**CO_2**	0.424	***< 0.001***	0.435	***< 0.001***	0.239	***< 0.001***	0.368	***< 0.001***	0.025	*0.228*	0.506	***< 0.001***	0.076	***0.036***
	CH_4	0.141	***0.002***	0.129	***0.004***	0.258	***< 0.001***	0.222	***< 0.001***	0.147	***0.003***	0.182	***< 0.001***	0.231	***<0.001***
Winter	**CO_2**	0.394	***< 0.001***	0.409	***< 0.0001***	0.062	*0.068*	0.035	*0.178*	0.041	*0.137*	0.361	***< 0.001***	0.013	*0.409*
	CH_4	0.356	***< 0.0001***	0.345	***< 0.0001***	0.005	*0.630*	0.001	*0.851*	0.003	*0.698*	0.195	***0.001***	0.119	***0.013***

Table 3.6: Results of linear models showing relationships between potential production of CO_2 and CH_4 and sediment explanatory characteristics, and the first and second principle components from the principle component analysis discussed previously.

3.3.3 Temperature dependency

The calculated activation energies are summarised in Table 3.7. Overall, the temperature dependency of CH_4 production was higher than that for CO_2 production (T_{12}=4.62, p<0.001). No significant differences were found between summer and winter, for either CO_2 or CH_4. Additionally, no differences were found between rivers or geology types. Instead, measurements of temperature dependency were relatively consistent, at around 0.50 eV for CO_2 and 0.96 eV for CH_4.

Given that the temperature dependency for CH_4 is considerably higher than that for CO_2, as well as an increase in the total mineralised carbon the proportion of mineralised carbon emitted as CH_4 increases with temperature. The slopes of natural logged-production rate vs. standardised temperature were used to estimate the magnitude of this effect. With an increase in temperature from 10 to 14°C, the amount of CO_2 produced increases by an average of 34%, whilst CH_4 production increases by 86%. Therefore, at 10°C, 6.7% of total carbon is mineralised to CH_4, whereas at 14°C this rises to 7.9% (T_{119}=6.26, p<0.001).

		Carbon Dioxide Activation Energy, eV				Methane Activation Energy, eV			
	River	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*
Chalk	Ebble	0.47	*0.03*	0.38	*0.09*	1.64	*0.33*	1.18	*0.44*
	Wylye	0.40	*0.11*	0.31	*0.09*	1.48	*0.09*	1.32	*0.50*
	Wylye Patch A	0.47	*0.06*	0.51	*0.03*	1.14	*0.11*	0.75	*0.04*
	Wylye Patch B	0.68	*0.16*	0.46	*0.07*	0.73	*0.30*	1.20	*0.23*
	Rushall-chalk	0.64	*0.03*	0.34	*0.07*	1.81	*0.29*	1.12	*0.47*
Sand	Avon	0.50	*0.06*	0.66	*0.19*	1.18	*0.27*	0.11	*0.21*
	Avon Patch A	0.44	*0.04*	NA	*NA*	1.01	*0.12*	NA	*NA*
	Avon Patch B	0.42	*0.05*	0.52	*0.05*	0.64	*0.18*	0.99	*0.26*
	Nadder	0.58	*0.21*	0.34	*0.02*	0.81	*0.56*	1.10	*0.38*
	Rushall-sand	0.33	*0.15*	NA	*NA*	1.14	*0.08*	NA	*NA*
Clay	Sem	0.51	*0.08*	0.83	*0.42*	0.64	*0.19*	0.19	*0.66*
	Priors	0.67	*0.06*	0.56	*0.11*	0.64	*0.19*	0.71	*0.38*
	Cools	0.58	*0.06*	0.45	*0.06*	1.18	*0.16*	0.42	*0.14*

Table 3.7: Calculated activation energies of carbon dioxide and methane production for each river, in summer and winter (*n=5*, standard errors in italics).

3.4 Discussion

Anaerobic potential CO_2 and CH_4 production was measured in anoxic riverbed sediments across nine rivers and three catchment geologies. Significant production of both CO_2 and CH_4 was measured, with highest rates in the clay and lowest in sand. When comparing intra-site variation, particularly high rates were found in the vegetated sediments of the Wylye (chalk) river, reflecting overall high variation in the chalk sites. Production of CO_2 was higher in summer than in winter across the rivers, reflecting changes in measured sediment characteristics; though CH_4 did not show a seasonal change. The response to temperature of CO_2 and CH_4 production was investigated, and were found to be consistent over geology and season. However, the response to warming of CH_4 production was consistently significantly higher than that of CO_2, indicating a shift in the carbon gas balance from CO_2 to CH_4 as temperature increases.

3.4.1 Sediment characteristics

The sediment characteristics varied between individual rivers but there were some similarities arising from underlying geology and riparian cover. Sediment chlorophyll content was highest in the chalk rivers and lowest in the clay rivers, reflecting the gradient in local macrophyte growth across the geologies. Macrophyte growth is predicted largely by solar radiation (Flynn *et al.* 2002), and the chalk rivers have both clearer water and are less shaded by tree cover. Higher chlorophyll concentrations were measured in summer than in winter, reflecting seasonal growth and dieback in macrophytes (Clarke 2002). However, organic carbon and nitrogen did not follow the same pattern as chlorophyll, with high concentrations found in both chalk and clay. This indicates that although there is little local primary production occurring in the clay rivers, they do have a source of particulate organic matter, most likely from lateral transport from surrounding terrestrial systems due to surface runoff as well as the increased input of nutrients due to preferential flow through tile drains (Laubel *et*

al. 1999). No overall difference in organic carbon content was measured between summer and winter, although the change in chlorophyll content indicates that a greater proportion of the organic matter present may be labile in summer; whilst in the winter it is older, more recalcitrant carbon.

Investigation of the particle size data, summarised using the median particle size (d50), confirms that the chalk sites have the largest particle sizes, whilst the lowest values are seen in the clay sites. However, it is important to note that there is a lot of variability present, such as the larger d50 values at Priors compared with the other clay sites. This is due to the large cobbles and stones present at this site along with the small clay particles, and is in agreement with what can be seen at the site. Whilst it has a high d50, Priors has the highest proportion of clay particles, with 23% of the sediment sampled in summer < 2 µm.

The largest variation within rivers, regarding particle size as well as other sediment characteristics, is seen in the chalk sites (Figure 3.1). When comparing the Wylye main bed sediment with the vegetated and marginal sediments, there are some noticeable differences. Particle size of the main bed is the highest measured, as it is bare gravel, whereas the vegetated and marginal patches have some of the lowest particle sizes of all the rivers. Other patch differences were apparent, with particularly high organic carbon content in the vegetated sediment (15.66% dry weight in winter). Although this does not correspond to a high chlorophyll content (0.23 µg g dw^{-1}): suggesting the organic carbon present may not be very labile.

This illustrates an important factor when considering metabolic rates between rivers: the variation in sediment characteristics between different patches and even individual samples was at times higher in the chalk rivers than variation between rivers or geology types. However, this was not seen in the Avon (the sand river that was also chosen to investigate intra-reach variation). The coarse, highly permeable chalk riverbeds may be increasing the time water remains in an area, and so organic carbon can be retained and so there is an opportunity

for aggregates to be formed (Battin *et al.* 2008). Additionally, macrophyte growth slows flow and so can cause the retention of organic matter (Sand-Jensen 1998), added to by the decomposition of macrophytes (Clarke & Wharton 2001); resulting in patches of nutrient-rich fine sediment. This was particularly apparent in the Patch A samples from the Wylye measured here; which recorded low particle diameter, high organic carbon content and correspondingly high rates of CO_2 and CH_4 production.

3.4.2 Production of carbon dioxide and methane

Rivers are typically considered as being less important sources of methane compared to wetlands, rice paddies and lakes (Ciais *et al.* 2013); as running water keeps the sediment more oxygenated. However, though lower than other freshwaters significant production does occur in rivers and streams, as shown in this study and others (Jones *et al.* 1995; Mach *et al.* 2015).

When considering the production of CO_2 and CH_4 with respect to geology type, there are some overall trends, for CO_2 at least, with higher production in the chalk and clay rivers. However it is clear that underlying geology alone is not sufficient to predict production. Instead, the first component (PC1) from the principal component analysis is a good predictor of CO_2 production, and both PC1 and PC2 are reasonable predictors of CH_4 production. However simply using organic carbon or nitrogen content also resulted in good predictions of both CO_2 and CH_4 potential production; these were the most important sediment characteristics measured in terms of CO_2 and CH_4 production rates. Geology is useful only where it correlates with sediment characteristics, for example clay tends to have more organic carbon (and thus more production) compared to sand. but the sediment characteristics reveal variation between and within rivers that is not accounted for by simply classifying by geology, such as fine sediment build up under submerged macrophytes in the chalk rivers, which is represented by the PCA. The organic carbon and nitrogen content, as well as particle size and $\delta^{13}C$ to a lesser extent, are useful indicators of potential CO_2 production.

The relationship between organic carbon and CH_4 production is not as strong as CO_2, which may be in part due to the known heterogeneity of methane production (Adrian *et al.* 1994; Wachinger *et al.* 2000). Methanogenesis is limited to 'hot spots' of anoxic sediment, in the absence of oxygen and other oxidants (nitrate, sulphate, ferric iron) (Conrad 2009). There is, however, a strong correlation between CO_2 and CH_4 production (r^2 = 0.50, p < 0.001), indicating that areas suitable for carbon catabolism overall are also most suitable for methanogenesis, where redox state allows.

Microbial activity by fluorescein diacetate hydrolysis is used as an estimate of microbial biomass pool size, and is expected to be related to CO_2 production (Schnurer & Rosswall 1982). A relationship was found, though not particularly strong (r^2=0.10, p = 0.0003). Agreeing with CO_2 production though, there is a higher rate of microbial activity in summer than in winter. The low r^2 may be an indication that the laboratory CO_2 experiments, with unavoidably disturbed sediments, do not reflect the true CO_2 production in situ (Sánchez-Monedero *et al.* 2008): an important consideration when using potential measurements in the laboratory rather than field-based experiments of undisturbed sediment.

As described above, large differences in sediment characteristics were found between patch types in some rivers, and sediment qualities such as organic carbon and particle size were important sources of variation in CO_2 and CH_4 production which could not always be accounted for by underlying geology alone. As shown in previous studies comparing vegetated and un-vegetated riverbed, the fine sediments and high organic matter content typical under macrophytes are sites of increased CO_2 and CH_4 production (Jones *et al.* 1995; Pinardi *et al.* 2009). As macrophyte cover can exceed 70% in chalk riverbeds (Cotton *et al.* 2006); this can significantly influence the amount of carbon mineralisation by the microbial community.

This is an important consideration when measuring discrete samples or areas of rivers. Whilst much of the Wylye is gravelled main bed, a significant proportion is covered in macrophytes which cause fine sediment build up (patch A in these experiments). The

experiments found that production of both CO_2 and CH_4 was far higher in the sediments of patch A than they were in the main bed. This is in agreement with other studies, that fine sediments or vegetated areas are sites of significant production (Jones *et al.* 1995; Pinardi *et al.* 2009; Shelley *et al.* 2015). Patch B (marginal sediment) had a high production of CO_2 and CH_4 in winter, though in summer it was approximately the same. This correlated with higher organic carbon in the winter. However, for the Avon, the differences were not seen: as the sediment characteristics were much more similar between main bed and the two patches measured than in the Wylye. This is in agreement with the idea that local sediment characteristics are more important than the larger geological catchment, and need to be considered when estimating reach-scale rates of metabolism. This is especially true for chalk rivers, which can have particularly heterogeneous riverbeds. Although only one river in the study included exploration of distinct patches, all the chalk rivers showed high variability in the sediment characteristics measured here.

3.4.3 Temperature dependency

Temperature dependence describes the strength of response to temperature of a metabolic process, and can be expressed as activation energy, measured in electronvolts (eV). The activation energies found here for CO_2 production (0.5 ±0.02 eV) are of a similar range to those published in other studies for lakes (0.44-0.65 eV, (Yvon-Durocher *et al.* 2012)), other rivers (0.44-0.78 eV ((Yvon-Durocher *et al.* 2012), and 0.53 ±0.12 eV (Acuña *et al.* 2008), and fine sediments in a chalk river (0.24 eV (Shelley *et al.* 2015). There were not any differences found between rivers, geology or season (Figure 3.6), and results are in agreement with the average close to that expected for heterotrophic metabolism (0.65 eV, (Yvon-Durocher *et al.* 2010b).

The activation energies calculated for CH_4 production (0.97 ±0.07 eV) are comparable to previous measurements in mesocosms (0.64-1.02 eV, (Yvon-Durocher *et al.* 2010b)), peat soils

(1.3-2.8 eV (Dunfield *et al.* 1993)), lakes (0.6-1.3 eV, (Duc *et al.* 2010) and 0.7-2.0 eV (Lofton *et al.* 2013)), and fine sediments in a chalk river (0.51 eV (Shelley *et al.* 2015). This is in agreement with a recent meta-analysis (Yvon-Durocher *et al.* 2014) which found a similar average temperature dependence of methanogenesis across a range of ecosystem types. As with CO_2, activation energies were constant across the different geologies and patch types; and no significant difference was seen between summer and winter incubations.

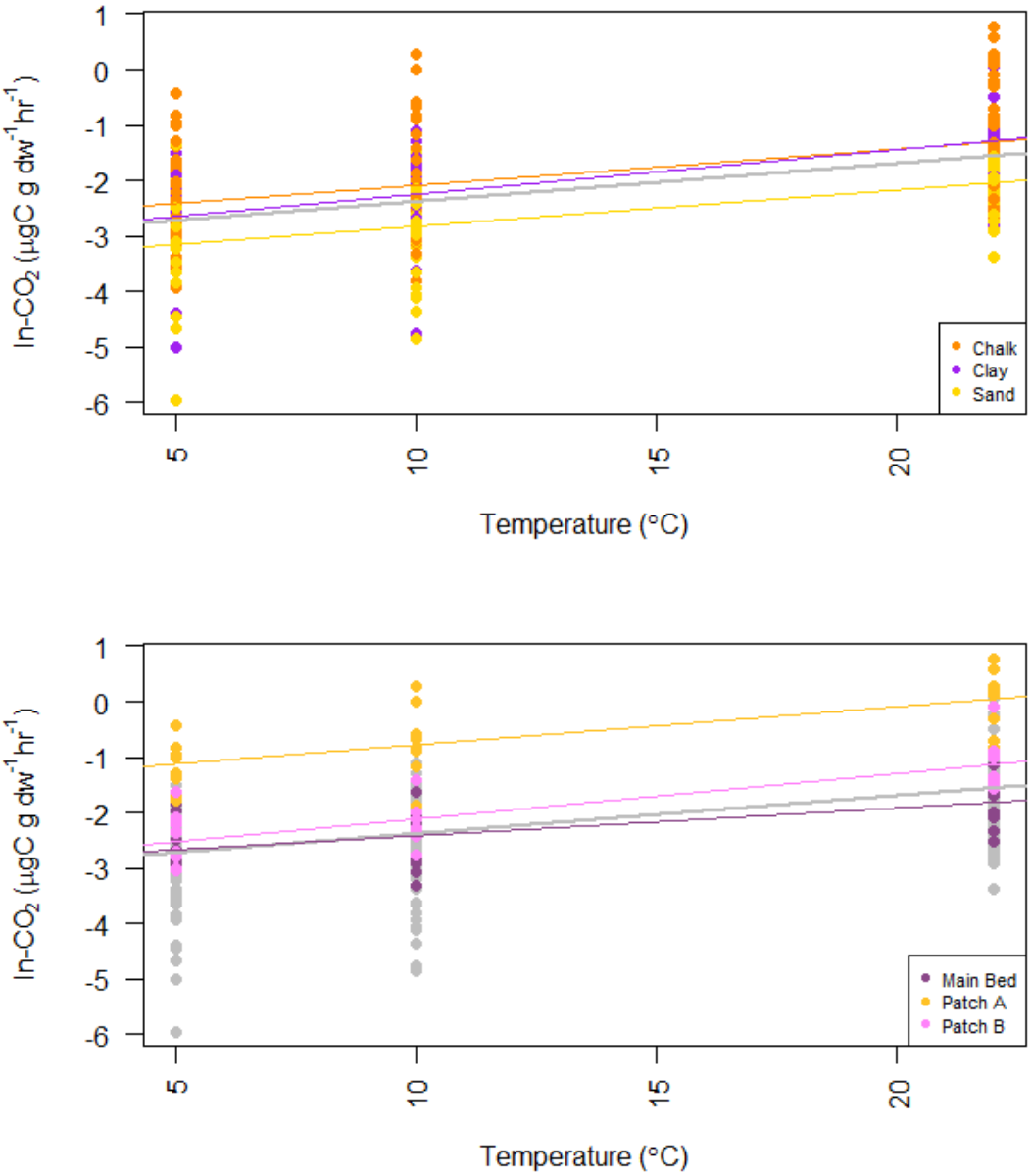

Figure 3.6: Relationship between incubation temperature and natural-logged CO_2 production. Shown in top panel, all samples separated by geology type. Parallel lines indicate consistent temperature dependency. Similar intercepts (for chalk and clay) represent similar overall production of CO_2 whilst lower intercept for sand indicates lower overall production. In bottom panel, Wylye samples are highlighted (other samples shown in grey). Again, response to temperature is similar, whilst large difference in intercept between the main bed and Patch A represents significant effect of patch on overall production. Difference between production in main bed and Patch A is greater than difference between production at 5°C and 22°C in the same patch.

In most terrestrial (Allen*et al*, 2005) and oceanic(del Giorgio and Williams,2005) ecosystems, respiration and methanogenesis are linked to local primary production of organic compounds; as they cannot respire more organic matter than is fixed locally. But in freshwater ecosystems this is not always the case due to the large lateral input of terrestrial organic matter (Cole & Caraco 2001). As such, they may be expected to respond more strongly to increases in temperature, being less restrained by substrate limitation than many other systems (Yvon-Durocher*et al,* 2010c). This suggests a possible positive feedback with increasing global temperatures due to climate change in these systems.

As found by others in mesocosms (Yvon-Durocher *et al.* 2010b) and a chalk river (Shelley *et al.* 2015); the temperature dependence of CH_4 production in this study is approximately double that of CO_2 production. By the end of the 21st century, global surface temperature is likely to exceed 2°C relative to the average for 1850-1900 for most modelling scenarios (IPCC 2013). The difference in temperature dependence measured here indicates not only an increase in total carbon mineralisation but also an increase in the proportion of carbon being mineralised as CH_4 rather than CO_2. This is significant when considering the impact on climate change as CH_4 has 28 times the global warming potential of CO_2 over 100 years (Myhre *et al.* 2013). Indeed, even at a temperature of 10°C this study predicts an average of 6.7% of carbon being mineralised as CH_4, which in terms of global warming potential means CH_4 emissions from these river sediments are already twice that of CO_2 emissions.

However, it should be noted that these experiments were carried out under anoxic conditions, and so do not take into account the oxidation of methane by methanotrophic bacteria *in situ*. Methanotrophy takes place in the oxic top layer of sediment, and does not respond to increases in temperature (Duc *et al.* 2010; Shelley *et al.* 2015) as it is often substrate limited (i.e. by availability of methane). This suggests that, with an increase in methanogenesis due to temperature, methanotrophy would respond to the increase in

available CH_4 substrate and so greater rates of CH_4 oxidation would occur, therefore a net increase in CH_4 emissions may not necessarily be seen (Duc *et al.* 2010).

3.4.4 Comparison of effects of temperature and sediment type on carbon mineralisation

These experiments have demonstrated the uniform, strong response to temperature of sediment CO_2 and CH_4 production is conserved across a gradient of geology and between varying sediment types (Figure 3.5), as well as in both summer and winter. However, also shown here is how the production of CO_2 and CH_4 can vary by an order of magnitude or more depending on the sediment characteristics. In the gravel of the main Wylye bed, CO_2 production at 10°C in winter was measured as 0.07 µg C g dw^{-1} hr^{-1}, compared to 0.53 µg C dw^{-1} hr^{-1} in patch A (vegetated sediment). This makes apparent how, in some cases, changes in land use may be more significant than changes in temperature: with even a 4°C rise in temperature to 14°C, the Wylye main gravel bed only produces 0.09 µg C g dw^{-1} hr^{-1}, nearly 6 times less than patch A produces at 10°C. Additionally, the proportion of carbon mineralised as CH_4 rather than CO_2 changes between river and sediment type (Table 3.3), with 11% CH_4 in the Wylye main bed compared to 51% in patch A in winter. The three sand sites consistently had the lowest proportion of carbon mineralised to CH_4, ranging between 0.15-1.36%.

Many rivers, including those in this study, are heavily influenced by anthropogenic activities such as farming and diversions or damming of water (Neal & Jarvie 2005). In particular, the delivery of sediment to rivers is affected by activities such as agriculture (Collins & Walling 2007); and has increased in recent years (Wood & Armitage 1999; Jones *et al.* 2012). Furthermore, eutrophication and especially the input of excess phosphorus to rivers from agricultural and industrial activities can increase the amount of macrophyte growth in rivers (O'Hare *et al.* 2010). In the context of these considerations, the results described in this chapter suggest that, although an increasing surface temperature may cause an increase in greenhouse gas production in riverbed sediments changes in land use have the potential to increase the rate of carbon mineralisation at a far greater magnitude. The fine sediments, rich

in organic matter, trapped by macrophytes in chalk rivers are ‘hotspots’ of carbon mineralisation, far in excess of the main riverbed in chalk rivers or those on other geological landscapes. Thus the increase in delivery of fine sediment and the increase in macrophyte growth to trap it may have profound effects which must be considered and included in future climate mitigation and land use strategies.

3.5 Conclusion

The data presented in this chapter have shown how sediment characteristics can vary with underlying geology, but also within individual river reaches. High organic carbon content is an important predictor for CO_2 and CH_4 production, but the origin of this could be both local production, in chalk systems, or allochthonous input, particularly in clay systems. Sand rivers in between these two extremes have lower organic matter and lower CO_2 and CH_4 production. This research is unique in incorporating both a range of geology types as well as different patches in individual rivers, allowing the controls and variation on production to be understood. The findings regarding temperature dependence support previous findings that activation energies are conserved across different systems, and imply a positive feedback with warming of a higher proportion of CH_4 mineralisation.

The findings in this chapter are taken forward in the next chapter, which uses *in situ* measurements to test how much the differences in carbon metabolism between geologies and patches persist under less disturbed conditions. Additionally, the patch studies are expanded to more rivers, and how diel cycles modulate CO_2 and CH_4 production are considered, together with further consideration of seasonal and temperature involvement.

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313.

4. How in situ carbon metabolism varies with geology, season and light.

4.1 Introduction

4.1.1 Background

The previous chapter discussed potential production of carbon dioxide (CO_2)and methane (CH_4) under laboratory conditions. Whilst useful, these measurements cannot be directly extrapolated to what is happing *in situ*, where temperature, discharge, diel cycles, redox state and variations in light would all be expected to influence rates of metabolism.

There are several ways to estimate rates of benthic metabolism in rivers and streams. Single or two station measurements of change in oxygen (O_2) concentration in the water can be used to estimate metabolism (Odum 1956; Uehlinger *et al.* 2002; Acuña *et al.* 2004). Alternatively, chambers which enclose a patch of riverbed together with overlying water can be used to measure the change in O_2 or CO_2 in the water, and so calculate the flux from the sediment (Boynton *et al.* 1981; Bott *et al.* 1984; Bunn *et al.* 1999). More recently, eddy correlation techniques, which combine flow measurements and high resolution O_2 data, have been applied to inland waters (Berg *et al.* 2013).

Measurements using chambers to enclose individual patches of sediment do have known disadvantages – fixing them to the sediment means the method is more invasive than others, and pore-water flushing and the normal movement of water over the sediment cannot be accurately represented (Berg *et al.* 2013). However, out of the three methods, only incubations using chambers can enclose small areas of riverbed, and thus make specific measurements of these areas rather than simply averaging the whole reach. This means that differentiation can be made between, for example vegetated and un-vegetated areas, or littoral zones and the main channel. Most studies do not incorporate this distinction, but the differences in fine sediment accumulation, light intensity and flow rate may be expected to affect rates of carbon metabolism. One study that compared the main channel of a freshwater

system with littoral zones found GPP rates ~10 times higher in the latter area (Bunn *et al.* 2003). The previous chapter in this study showed that, when patches were considered, CO_2 production was 5 times higher in vegetated sediment, and CH_4 more than 50 times higher, compared with the main chalk bed.

So including consideration of different patches within a reach may be needed in order to estimate whole reach scale metabolism, and using benthic chambers can supply valuable information about the difference in metabolism between differing microhabitats within a reach, as well as comparing various whole-reach measures.

Methane production has been studied less extensively in rivers; and rice paddies and natural wetlands and are understood to be the largest natural source of CH_4(Kirschke *et al.* 2013). In fact the main bed (i.e. gravel) in chalk rivers may be a net CH_4 sink due to oxidation by methanotrophs (Trimmer *et al.* 2010). However ingress of fine particulate material, even in rivers of permeable sediments such as chalk, can cause development of conditions anoxic enough that significant methanogenesis occurs (Sanders *et al.* 2007). Additionally, those rivers and streams on impermeable catchments may be hypothesised to be sites of strong methanogenesis due to anoxia and high allochthonous carbon input.

As carbon metabolism is affected by temperature, light, and substrate availability among other things, and these will vary with season, it should be apparent that metabolism will change throughout the year. Therefore when measuring these processes it is important to repeat experiments throughout the year in order to fully describe the contribution of streams on contrasting geologies to carbon budgets.

Furthermore, as described previously in Section 1.3, the underlying geology and resultant base flow index (measure of the proportion of water originating from groundwater) may affect carbon metabolism of the benthic sediment. When explored in Chapter 3, considering the study rivers by their underlying geology revealed some differences in sediment properties and carbon metabolism. These considerations will be investigated further in this Chapter.

4.1.2 Outline of Chapter

This chapter is concerned with determining the actual rates of respiration, gross primary production, net ecosystem metabolism and methane production in the sediments of the study sites, expanding on the potential measurements described in chapter 3. This was achieved using benthic chambers to enclose a small section of sediment with the overlying water, and measuring the change in O_2, CO_2 and CH_4 in thechambers. Repeating these measurements during all four seasons, using both dark and light chambers, as well as measuring fluxes from different sediment types ('patches'), ensures a complete estimate of carbon metabolism for the sites.

The outcome of these experiments, combined with those of the previous chapter, are used to illustrate whether benthic metabolism is affected by geology, as well as exploring other sources of variation in rates of production. Furthermore these results are necessary for determining local CO_2 and CH_4 production, for later discussion on the proportion of out-gassing accounted for by riverine metabolism.

4.1.3 Objectives:

- To measure rates of CO_2 and CH_4 production, along with O_2 consumption, under natural conditions.
- To map the reaches of river where the experiments are carried out in order to get an accurate measure of the areal extent of different patch types (vegetated, marginal fine sediments and the main gravel or sand riverbed) over the whole reach.
- To use the results above together with light measurements to calculate whole reach rates of ER, GPP, NEM and CH_4 production.

- To explain how these results are affected by seasonal differences, as well as across different patch types and geologies which give rise to a gradient of BFI.

4.2.1 Study site description

These experiments were carried out on the six main rivers described in Section 2.3. Two rivers are on a chalk catchment, two on sand and two on clay. Properties of each river are summarised in Table 2.1. As described previously, these rivers encompass a range of base flow index (BFI), which is indicative of the proportion of water derived from groundwater.

As discussed in Section 2.3, heavy rainfall and flooding occurred during autumn and winter 2013-2014, such that some experiments could not be undertaken in all rivers. Consequently, data for the Avon (sand) and Ebble (chalk) are incomplete.

4.2.2 Experimental design

Measurements of *in situ* O_2 consumption, CO_2 and CH_4 production were carried out using small benthic chambers (Figure 4.1) secured in the river bed. The chambers consisted of three sections, each which was installed separately. First, the bottom, steel collar was pushed into the sediment, approximately 10cm until the top lay flush with the sediment surface. This was then left for 30 minutes so any disturbance caused by installing the collar was reduced. The middle section of the chamber was then fixed onto the collar using screws, which were tightened by hand. A watertight seal was ensured due to rubber o rings between the different sections of the chamber. The top of the chamber, containing sampling valves, space for a magnetic stirrer and O_2 sensors was then screwed on, after brushing them underwater to remove presence of air bubbles.

These chambers isolated 73cm^2 of the river bed, with 500ml of overlying river water. Magnetic stirrers were used to agitate the water and keep the oxygen concentration homogeneous. Potassium chloride was added as a tracer, to measure potential exchange of the water between the chamber and surrounding river and so detect any leaking chambers.

Light intensity (lumens m^{-2}) and temperature (°C) were measured withHOBO Pendant Data Loggers which were attached to the chambers (Tempcon Instrumentation Ltd. West Sussex, UK).

Both light and dark chambers were used. Light chambers, made from steel and Perspex, were used to measure NEP and gas production in the light (i.e. daytime), whilst dark chambers, made from steel and a black plastic, measured respiration as well as gas production in the dark (i.e. night-time). Four chambers were deployed at a time, for approximately two hours. Each study site was visited for three days during each campaign, and two deployments were carried out each day giving a total of 12 light and 12 dark measurements per site per seasonal campaign. The light and dark measurements were paired, and where possible a range of sediment types were measured during each campaign (e.g. under vegetation and in areas of marginal, fine sediment as well as the main bed).Vegetation was not included within the chamber; and subsequently results describe metabolism of the sediment microbial community and do not include macrophyte metabolism. Where necessary, plants were cut back to allow access to the riverbed.

The consumption of O_2 in the chambers was measured using oxygen microsensors connected to an UnderWater Meter (Unisense, A/S, Denmark). The sensors were connected through the top of the chambers and sealed with a cable gland. They measured O_2 once a minute throughout the duration of each deployment, and the data was logged by the UnderWater Meter. CO_2 and CH_4 were measured by taking water samples from the chambers at the start and the end of the deployment, to be measured by gas chromatography at a later date. Samples were taken using a plastic syringe connected to a valve on top of the chambers. Balloons in the chambers ensured that taking these samples did not allow the water inside of the chambers to become pressurised. The samples for CH_4 were put in 12mL gas-tight vials (Exetainers, Labco, UK), and overfilled to ensure no air was introduced. The samples for CO_2 were taken according to the method described in Section 4.2.4.

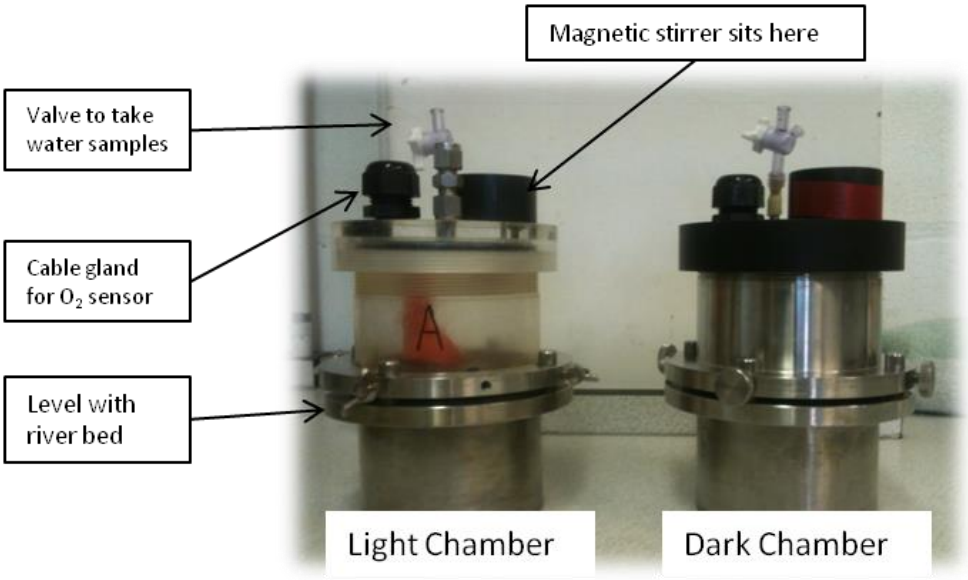


Figure 4.1: Light and dark benthic chambers used for *in situ* incubations.

4.2.3 Mapping

During each seasonal campaign physical mapping of each study site riverbed was carried out following methods described in Gurnell *et al*.(1996). Scale maps of approximately 200m reaches of the rivers were downloaded from Digimap (Edina, University of Edinburgh, UK). The physical characteristics of the riverbed such as sediment type and vegetation cover were drawn onto the maps by hand whilst walking along the river. These maps were scanned and colour was overlaid onto the digital copies. Photoshop was then used to calculate what percentage of each map was covered by each colour, i.e. each physical characteristic type. The Photoshop portion of this analysis was carried out by Lorenzo Rovelli. This data could then be used firstly to examine how the riverbed characteristics such as vegetation cover changed between sites and seasons. Secondly, it was used together with the benthic chamber data to scale-up the results for reach-scale estimates of metabolism (see Section 4.2.5).

4.2.4 Laboratory analysis

The O_2 measurements carried out with the microsensors output results in millivolts (mV). These were converted to millimoles per litre using Winkler titrations of a 100% saturated river water sample and measuring an oxygen-free solution of sodium hydroxide and sodium ascorbate with the microsensors in the field, to generate a calibration curve. This could then be used to convert the *in situ* O_2 mV measurements to millimolar concentrations.

The 12mL water samples for CH_4 were preserved within two hours of being taken by adding 100µl of 50% zinc chloride solution. The 3mL water samples for CO_2 were gas equilibrated on site by introducing 3mL of oxygen-free nitrogen and shaking by hand for 2 minutes. The gas was then stored by displacing degassed deionised water from 3mL gas tight vials (Exetainers, Labco, UK).

The CH_4 and CO_2 samples were analysed using gas chromatography according to the method described in Section 3.2.3.The CO_2 results were not used in the data analysis however: due to lack of precision of the method and the data generated it was decided that O_2 only would be used as a proxy for respiration and primary production.

4.2.5 Data analysis

After calibration of oxygen measurements, the rate of O_2 consumption over time could be calculated. For the CO_2 and CH_4 results, there were just the two data points, at the start and end of each incubation used to estimate the rate. The dimensions of the chambers were then used to convert the change in gas concentration from that in the chamber to the amount in mmol m^{-2} hr^{-1}.

Linear mixed effects models were designed using R and the nlme package (R Core Team 2015; Pinheiro et al. 2015) to investigate the effect of geology, season, light and patch on O_2 and CH_4 production in the chambers. It was not possible to log transform the data due to a significant number of negative flux results; however, a weighting term was included in the CH_4

data to account for the non-equal variances across groups. Factors that were not of interest in a model were added as random effects. ANOVAs were carried out to assess the significance of each factor.

The O_2, and CH_4 results were then scaled-up using the mapping data. Because there were measurements taken in various sediment types with the benthic chambers, these could be compared with the percentage cover of that sediment type and so an estimate of total reach metabolism could be calculated, taking into consideration the differing amounts of each sediment type. Estimates of ecosystem respiration (ER), gross primary production (GPP) and net ecosystem metabolism (NEM) could then be calculated for each of the reaches, during each of the seasons.

Ecosystem respiration was calculated by multiplying the rates of O_2 consumption per hour in the dark chambers by 24 to get daily estimates. It was assumed that ER was constant throughout the day.

Gross primary production was calculated by subtracting ER measured in the dark chambers from the O_2 consumption measured in the light chambers; and multiplying by the hours of daylight at the river on the day of measurement. The results assume that GPP was constant throughout the hours of daylight through the day: however, this will be a simplification. The benthic chamber measurements were usually carried out between the hours of 10:00am and 12:00pm. In reality, GPP would likely be lower just after sunrise and just before sunset, and peak in the middle of the day. Because measurements were taken at similar times of the day, comparisons between days and rivers should not be affected by this, but it may add a source of error to the daily estimates of GPP described in the results.

The estimate of daily NEM was calculated by subtracting GPP from ER. Positive NEM values are seen when the systems are net heterotrophic, whilst negative values indicate net autotrophy.

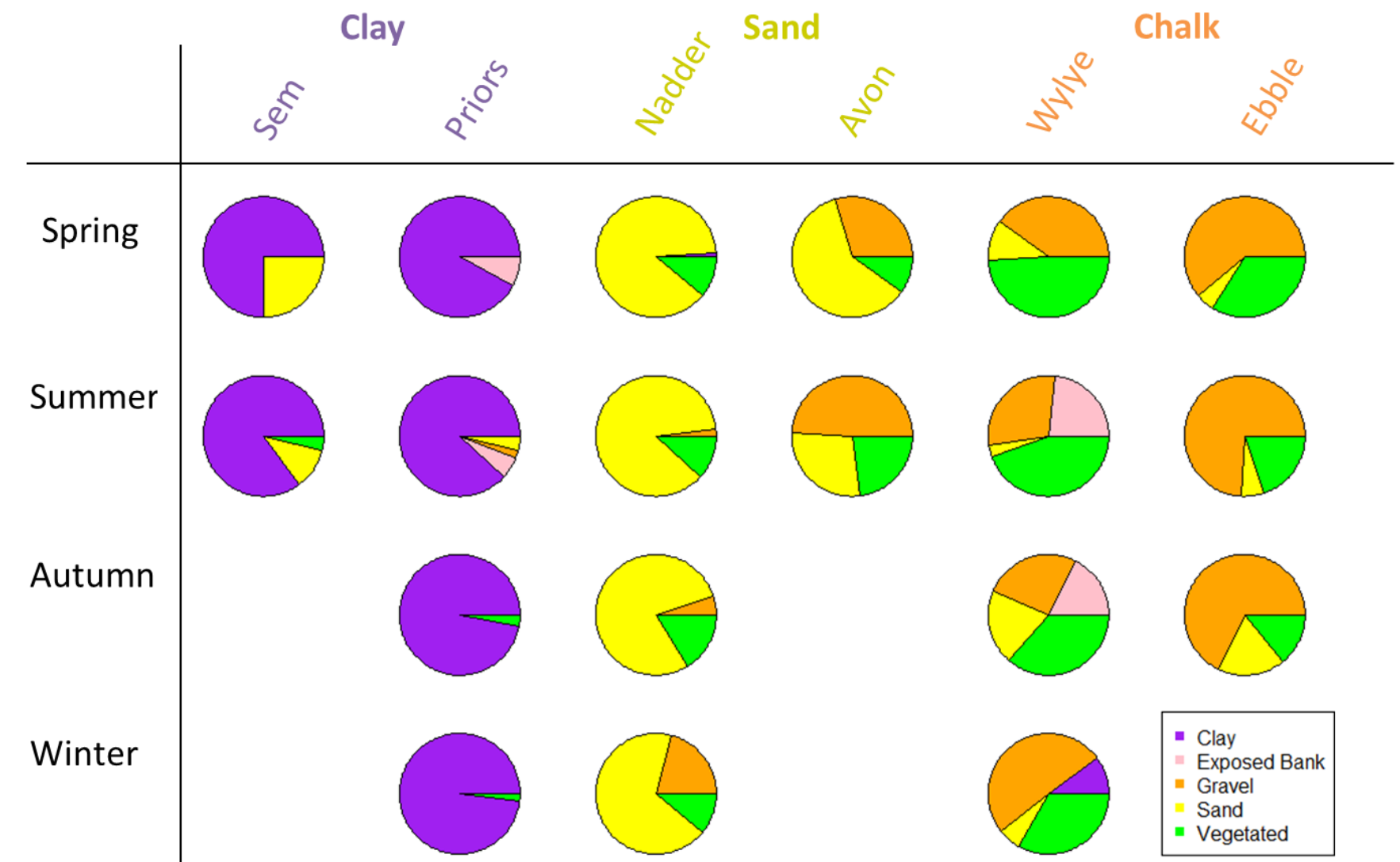
4.3.1 Mapping

Figure 4.2: Example of map used to scale up benthic chamber measurements. (Ebble (chalk), Spring campaign) Shows areas of main gravel bed (grey), areas covered by aquatic vegetation (green) and areas of fine, marginal sediment (yellow).

Mapping was carried out each season as illustrated in Figure 4.2, and the complete set of maps are included in the appendix, Section 7.2.The results of this exercise are summarised in Figure 4.3. It was not possible to map the Sem (clay) or Avon (sand) in autumn or winter, or the Ebble (Chalk) in winter, due to local flooding and high water levels in the rivers. Results show that the clay sites (Sem and Priors) were least heterogeneous, being mostly covered with clay; although the Sem did have some areas of sand/fine sediment and Priors had some gravelled areas, though these were very little of the total riverbed. Very small amounts of vegetation

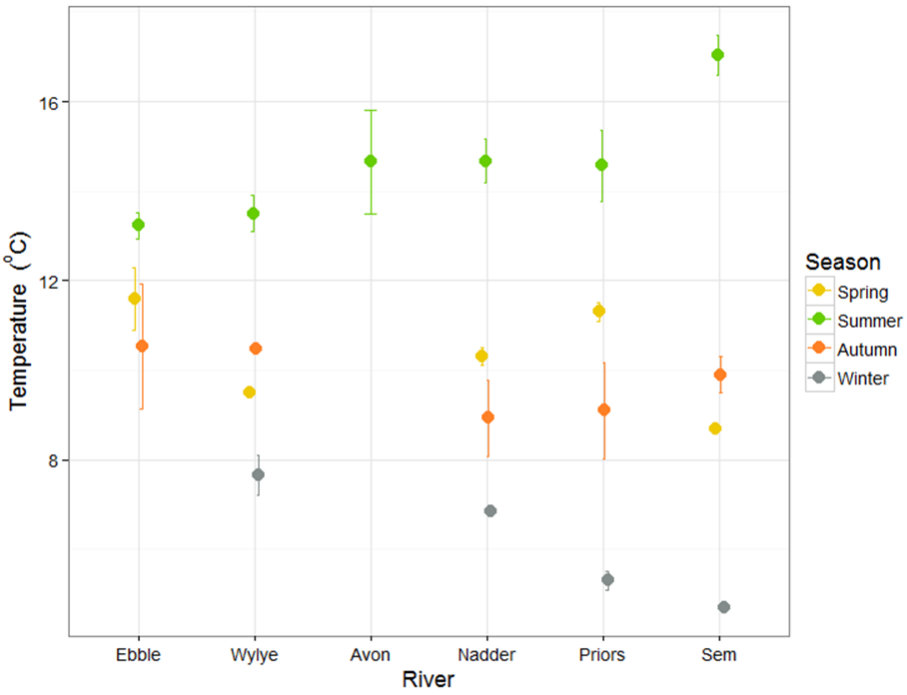
were evident at both sites. During spring and summer, areas of the riverbed of Priors were not covered by water due to very low flow, leaving exposed riverbed. The small amount of vegetation seen in Priors in the autumn and winter are thought to be terrestrial plants, which had been recently submerged by heavy rainfall and sudden changes in river level. The Nadder was the site with the highest proportion of sand, whilst the other sand site, the Avon, also had large areas of the riverbed that were covered in gravel. The Nadder had a higher proportion of gravel in autumn and winter compared to spring and summer, due to the high flow conditions causing sands to be scoured away and revealing gravels. Both the Nadder and Avon had a significant amount of vegetation cover. For the Nadder, this was highest in the autumn. The Wylye (chalk) was the site with the highest vegetation cover, and the other chalk site (Ebble) also had significant vegetation. However the highest levels for these sites were seen in spring and summer, unlike the Nadder.

During field campaigns, light and temperature were measured in the water at the sediment surface. All rivers had a similar average yearly temperature of around 11°C (Figure 4.4), with the highest temperature in summer and lowest in winter. However, the range between seasons varies with BFI. The Sem (clay), with the lowest BFI, had the largest variation in temperatures: from 4.7°C in winter to 16.6°C in summer. The Wylye, a chalk site with a high BFI, had a much narrower temperature regime with 7.7°C in winter and 13.5°C in summer. Light measurements were taken in the water close to the riverbed during the benthic chamber deployments. These are summarised in Figure 4.5, and show most light in the Ebble and Wylye (chalk), as well as in the Nadder (sand); with seasonal variation apparent. The clay sites show very low light intensity, and no variation between the seasons.



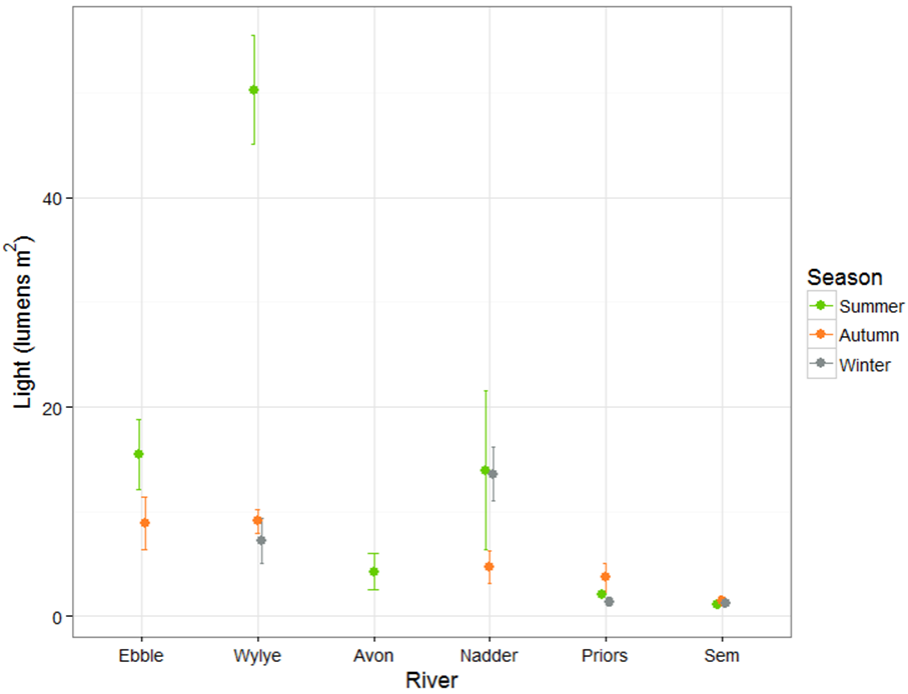
	Sem	Priors	Nadder	Avon	Wylye	Ebble
BFI HOST	0.234	0.372	0.695	0.861	0.931	0.953
BFI	0.207	0.551	0.814	0.732	0.885	0.905

Figure 4.3: Summary of results of riverbed mapping. Pie charts show the proportion of each river covered by each patch type. It was not possible to map the Sem or Avon in autumn or winter, or the Ebble in winter, due to weather conditions.



BFI	0.91	0.89	0.73	0.81	0.55	0.21
BFI-HOST	0.95	0.93	0.86	0.70	0.37	0.23

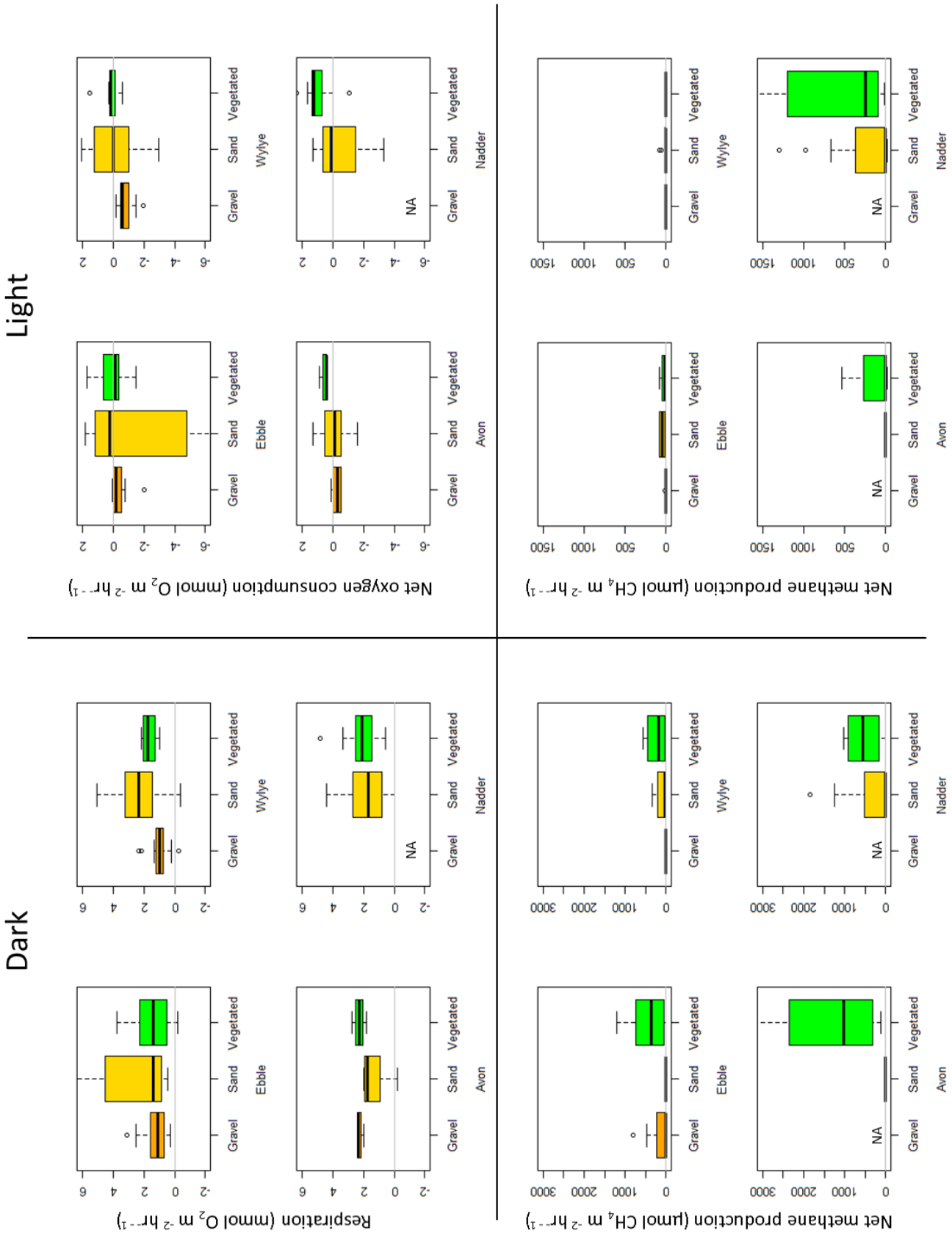
Figure 4.4: Temperature of the water in each river during each seasonal campaign. Rivers are ordered by BFI-HOST, from highest to lowest.



BFI	0.91	0.89	0.73	0.81	0.55	0.21
BFI-HOST	0.95	0.93	0.86	0.70	0.37	0.23

Figure 4.5: Light intensity of each river during seasonal campaigns. Measurements taken at close to riverbed, where benthic chambers were deployed. No measurements of light were carried out during the spring field campaign.Rivers are ordered by BFI-HOST, from highest to lowest.

Figure 4.6 Production of methane and consumption of O_2, comparison between patches in the sand (Avon and Nadder) and chalk (Ebble and Wylye) sites, in both dark and light chambers. No gravel measurements were available for the Nadder; or for methane for the Avon in gravel. Grey horizontal lines at zero indicate no net production or consumption. Negative values indicate production of O_2 or methane.



4.3.2 Oxygen, carbon dioxide and methane flux results

The results of the benthic chamber incubations are shown in Table 4.1. This table shows the average consumption of O_2 and production of CH_4 for each river, in each river. Both dark and light chambers are included: for O_2 the dark chamber measurements represent ER only, whilst the light chamber measurements are the net consumption of O_2 with both photosynthesis and respiration taking place.The CH_4measurements represent the net effect of methanogenesis and methanotrophy in both light and dark chambers.

Statistical analysis of the flux results was carried out to find if there were differences between the measurements. Results from the benthic chamber measurements of gas flux are considered first by chamber, river and season, then patch scale differences are investigated. The results of these analyses are summarised in Table 4.2. Lastly, the results are scaled up using the mapping data described above, to find estimates of reach scale carbon metabolism.

Mixed effects models were used to find the effect of light on CH_4 and O_2 metabolism by comparing the light and dark chambers, whilst keeping river, season and patch as random effects. Both oxygen ($F_{1,355}$=105.62, p<0.0001) and methane ($F_{1,265}$=5.38, p=0.021) flux were significantly affected by light. Oxygen consumption in the dark chambers was 1.78 mmol m^{-2} hr^{-1} on average, and in the light 0.331 mmol m^{-2} hr^{-1}. Methane production was higher in the dark chambers, at 612 µmol m^{-2} hr^{-1} compared to 281µmol $m^{-2}h^{-1}$ in the light.

Further analysis of oxygen consumption was carried out on two separate datasets: light and dark. This was due to the large differences between the two. Methane flux analysis was done on the dataset as a whole, conserving chamber as a random effect, due to the smaller significance of the difference between light and dark and the heterogeneity of the methane flux data.

The effect of season on O_2 consumption was significant in the light chambers ($F_{3,182}$=7.28, p=0.0001) but there was no difference found in the dark chambers. Oxygen consumption was

highest in the autumn (0.75 mmol m^{-2} hr^{-1}) and winter (0.71 mmol m^{-2} hr^{-1}). In spring, the net change in oxygen was negative, i.e. there was net production of 0.32 mmol m^{-2} hr^{-1}overall.

When the effect of season on CH_4 flux was tested, there was no significant difference between seasons.

Differences in flux between the rivers were also tested. River was a significant predictor of both O_2 consumption in the light ($F_{5,182}$=7.20, p<0.0001) and CH_4 ($F_{5,270}$=3.65, p=0.003) but not O_2 consumption in the dark. Oxygen consumption was highest in the clay (Sem 1.21 mmol m^{-2} hr^{-1} and Priors 1.01mmol m^{-2} hr^{-1}); and lowest in the chalk rivers which both measured net production of O_2 overall (Wylye 0.10 mmol m^2 hr^{-1} and Ebble 0.31mmol m^2 hr^{-1}). Methane production was lowest in the two chalk sites (Wylye 141. µmol m^2hr^{-1} and Ebble 94.9µmol m^{-2} hr^{-1}) and highest in the Nadder (sand, 683 µmol m^{-2} hr^{-1}).

	River	Season	Oxygen consumption, mmol m^{-2} h^{-1}				Methane production, µmol m^{-2} h^{-1}			
			Dark		Light		Dark		Light	
			Mean	*Std Error*	Mean	*Std Error*	Mean	*Std Error*	Mean	*Std Error*
Chalk	Ebble	Spring	0.97	*0.12*	-1.30	*0.77*	NA	*NA*	NA	*NA*
		Summer	2.33	*0.37*	-0.09	*0.22*	490.55	*167.85*	14.98	*10.21*
		Autumn	1.53	*0.73*	0.38	*0.27*	90.17	*52.02*	17.23	*9.37*
		Winter								
	Wylye	Spring	1.60	*0.16*	-0.48	*0.25*				
		Summer	1.43	*0.55*	-0.88	*0.25*	126.91	*55.19*	2.93	*1.22*
		Autumn	2.97	*0.93*	0.89	*0.73*	400.53	*365.87*	2.79	*13.45*
		Winter	1.15	*0.50*	0.12	*0.18*	-0.01	*0.21*	-0.11	*0.06*
Sand	Avon	Spring	1.98	*0.20*	-0.46	*0.20*				
		Summer	1.68	*0.41*	0.60	*0.23*	766.65	*457.87*	72.90	*77.46*
		Autumn								
		Winter								
	Nadder	Spring	2.54	*0.21*	-1.64	*0.31*				
		Summer	1.94	*0.45*	0.91	*0.18*	752.37	*365.30*	658.84	*351.27*
		Autumn	1.59	*0.43*	0.60	*0.22*	557.41	*474.52*	54.61	*20.65*
		Winter	1.26	*0.29*	0.88	*0.18*	729.96	*186.26*	1724.17	*1041.47*
Clay	Priors	Spring	1.34	*0.12*	1.81	*0.18*				
		Summer	2.54	*0.35*	0.99	*0.26*	1853.55	*835.49*	385.12	*210.41*
		Autumn	1.60	*0.21*	0.53	*0.28*	351.71	*317.16*	470.62	*575.56*
		Winter	1.13	*0.32*	1.02	*0.19*	616.79	*363.73*	25.57	*23.77*
	Sem	Spring	1.48	*0.21*	0.64	*0.59*				
		Summer	2.28	*0.27*	1.50	*0.10*	811.26	*693.91*	121.95	*61.53*
		Autumn	0.53	*0.29*	1.39	*0.25*	1067.10	*469.49*	143.96	*83.01*
		Winter	1.42	*0.50*	1.15	*0.43*	587.00	*449.35*	813.87	*822.73*

Table 4.1: Results of benthic chamber metabolism experiments. Results are not weighted by patch, and include all measurements at each river for each season. The O_2, rates are measurements of consumption, i.e. net respiration. CH_4 rates are measures of production, i.e. negative numbers indicate net consumption of the gas during the incubation.

4.3.3 Patch differences

The clay sites were not included in the analysis of patch-scale differences, due to the lack of vegetation and their relatively homogeneous riverbeds. Oxygen, again, was considered as two datasets, dark and light; whilst methane data were considered as one dataset with chamber (light or dark) as a random effect. Methane production was affected by patch type ($F_{2,145}$=8.89, *p*=0.0002), with the lowest fluxes in gravel (38.5 µmol m^2 hr^{-1}) and highest in vegetated patches (568 µmol m^{-2} hr^{-1}). Sand and marginal sediments were intermediate. Oxygen consumption was also lowest in the chalk (dark 1.23 mmol m^{-2} hr^{-1}, light net production of 0.42 mmol m^{-2} hr^{-1}) and also highest in the vegetated patches (dark 1.89 mmol m^{-2} hr^{-1},light 0.42mmol m^{-2} hr^{-1}).

Insufficient numbers of replicates, together with the considerable heterogeneity of methane fluxes, meant it was not possible to investigate the interaction between river and other factors. However, it appears that there may be differences in production between vegetated and un-vegetated patches between the sand and chalk rivers, with a greater difference between patches in the sand compared to the chalk (Figure 4.6). Conversely, the difference between methane flux in the light and dark chambers may be a greater difference in the chalk sites than it is in the sand.

Dependent variable	Fixed effect	Random effect(s)	*df*	F	*p*	Intercept
O_2	Chamber	Season, River	1,355	105.62	<0.0001	Dark: 1.78
						Light: 0.33
O_2 (dark)	Season	River	3,177	2.02	0.1131	Autumn: 1.81
O_2 (light)	Season	River	3,182	7.28	0.0001	Spring: -0.71
						Summer: 0.44
						Autumn: 0.75
						Winter: 0.71
O_2 (dark)	River	Season	5,177	0.43	0.8297	Avon: -1.81
O_2 (light)	River	Season	5,182	7.20	<0.0001	Ebble: -0.31
						Wylye: -0.10
						Avon: 0.30
						Nadder: 0.08
						Priors: 1.01
						Sem: 1.21
O_2 (dark)	Patch	Season, River	2,102	6.21	0.0029	Gravel: 1.23
						Sand: 2.08
						Vegetated: 1.89
O_2 (light)	Patch	Season, River	2,104	12.42	<0.0001	Gravel: -0.42
						Sand: 0.23
						Vegetated: 0.42
CH_4	Chamber	Season, River	1,265	5.38	0.0211	Dark: 612.4
						Light: 280.7
CH_4	Season	River, Chamber	2,267	1.05	0.3505	Autumn: 300.4
CH_4	River	Season, Chamber	5,270	3.65	0.0033	Ebble: 141.5
						Wylye: 94.9
						Avon: 356.7
						Nadder: 683.2
						Priors: 625.6
						Sem: 538.3
CH_4	Patch	Season, River, Chamber	2,145	8.89	0.0002	Gravel: 38.5
						Sand: 390.1
						Vegetated: 567.9

Table 4.2: Summary of modelling results, comparing oxygen and methane flux with season, river, chamber and patch. Units for O_2 consumption results (intercepts) are mmol m^{-2} hr^{-1}. Where negative, values indicated net production of O_2. Units for methane production are µmol m^{-2} hr^{-1}.

4.3.4 Reach scale metabolism

The data from the benthic chamber deployments, together with the mapping results were used to estimate reach-scale metabolism as shown in Table 4.3. Also shown are estimates of GPP, ER and NEM, calculated from the O_2 measurements in the dark and light chambers. Oxygen was used as a proxy for ER and NEM rather than CO_2 due to the availability of more accurate, higher frequency measurements of O_2 and being unable to collect CO_2 data for several rivers and seasons.

As shown in Figure 4.7a, little difference in ER was measured between sites overall, when measured at the reach scale. However, there were seasonal variations (shown in Figure 4.7c), with respiration highest in summer at 47.9±4.4 mmol O_2 m^{-2} d^{-1}, on average, and lowest in winter at 23.4±4.6 mmol O_2 m^{-2} d^{-1}. The overall pattern in ER was mostly consistent in each river, although the Nadder and Avon (the two sand sites) had higher rates in the spring than in summer (Figure 4.7e). The Wylye was the most inconsistent compared to the overall trend, with very high ER measured during the autumn, followed by spring and summer, with winter the lowest with only approximately a fifth the respiration rate of autumn.

Rates of GPP were more variable between rivers than ER. Shown in Figure 4.7b and f, the clay sites have very low, close to zero production, whilst the chalk and sand sites have rates which predominantly lie between 20 to 30mmol O_2 m^{-2} d^{-1}. When all rivers are aggregated (Figure 4.7d), the highest GPP is seen in spring with 26.7 ±10.3 mmol O_2 m^{-2} d^{-1}, and the summer is very similar with 22.7 ±3.7 mmol O_2 m^{-2} d^{-1}. GPP measured in the autumn and winter were much lower, with an average of only 2.9 ±1.3 mmol O_2 m^{-2} d^{-1} in the winter. Again, this pattern is largely consistent across the sites.

Rates of respiration were almost always higher than rates of GPP, and consequently calculations of NEM (Table 4.3) show the sites were net sources of CO_2 to the atmosphere. Over periods of 24 hours, the only times GPP exceeded ER were in the Ebble (chalk) and Nadder (sand) in spring, and these only with relatively low values of 4.08 and 5.78 mmol O_2 m^{-2}

d^{-1} respectively. Due to their low rates of GPP, the clay sites (Priors and Sem) had the highest rates of NEM, and so, potentially, were the largest sources of CO_2.

The relationship between respiration and primary production was tested (Figure 4.9a); to test how the presence of local, autochthonous primary production effected respiration. A significant positive relationship was found (r^2=0.38, p=0.002). Additionally, the water temperature of the sites was plotted against reach scale respiration (Figure 4.9b). The respiration data were log transformed and plotted against standardised temperature, $(1/(k/T))-(1/(k/T_c))$, where T is the temperature in Kelvin and k is the Boltzmann constant, 8.6173324 x 10^{-5}, and $1/(k/T_c)$ is the mean standardised temperature (Figure 4.9c). The relationship was significant (r^2 = 0.21, p = 0.025). The data were used in order calculate the temperature dependence, (as in Chapter 3), of 0.49 eV. Similar analysis was carried out to test the relationship between primary production and temperature (Figure 4.9d); no significant relationship was found.

The sites were consistently net sources of CH_4, rather than sinks, with the one exception of the Wylye during winter. There was large variation between sites and seasons, with patch-weighted, reach-scale estimates ranging from 0 µmol CH_4 m^{-2} hr^{-1} in the Wylye, in winter, up to 2420 µmol CH_4 m^{-2} hr^{-1} in the Nadder, also in winter; but the highest rates of production, on average, were found in the Nadder (sand) and Priors and Sem (both clay). The chalk sites (Ebble and Wylye) had the lowest measured rates of reach scale methane production, with the final sand site, the Avon producing an intermediate amount. As with the individual chamber results, there was a clear difference between the light and dark reach scale estimates of CH_4 metabolism, with approximately twice as much CH_4 produced in the dark compared to the light overall (Figure 4.8); although this varied widely between sites and seasons and the mean difference is poorly parameterised.

No relationship was found between CH_4 production and primary production or respiration, although there was a positive correlation between CH_4 production in the dark and net ecosystem metabolism (r^2= 0.29, p=0.023).

Geology	River Name	Season	O_2 (Dark)	O_2 (Light)	GPP	ER	NEM	CH_4 (Dark)	CH_4 (Light)
			mmol m^{-2} h^{-1}		mmol m^{-2} d^{-1}			µmol m^{-2} h^{-1}	
Chalk	Ebble	Spring	-0.97	0.81	27.39	23.31	-4.08		
		Summer	-2.16	0.27	37.60	51.92	14.33	390.10	8.20
		Autumn	-1.30	-0.19	11.96	31.15	19.19	32.80	10.78
		Winter							
	Wylye	Spring	-1.51	0.15	24.62	36.32	11.71		
		Summer	-1.33	0.45	27.03	31.95	4.92	143.70	2.72
		Autumn	-2.41	-0.34	22.74	57.76	35.03	276.13	2.13
		Winter	-0.53	0.14	6.64	12.83	6.19	0.00	-0.02
Sand	Avon	Spring	-1.82	0.53	36.95	43.60	6.66		
		Summer	-1.62	-0.61	16.14	38.87	22.73	603.41	57.15
		Autumn							
		Winter							
	Nadder	Spring	-2.58	1.71	67.72	61.93	-5.78		
		Summer	-2.05	-0.72	21.13	49.15	28.03	802.18	372.28
		Autumn	-1.32	-0.47	8.84	31.78	22.94	321.85	44.84
		Winter	-0.82	-0.72	1.03	19.60	18.57	692.96	2419.74
Clay	Priors	Spring	-1.34	-1.81	-6.65	32.28	38.92		
		Summer	-2.54	-0.99	22.15	61.03	38.88	1853.55	385.12
		Autumn	-1.60	-0.53	10.79	38.49	27.69	351.71	470.62
		Winter	-1.13	-1.02	1.14	27.17	26.03	616.79	25.57
	Sem	Spring	-1.48	-0.81	10.26	35.52	25.27		
		Summer	-2.28	-1.50	12.04	54.72	42.68	811.26	121.95
		Autumn	-0.53	-1.39	-8.58	12.64	21.22	1067.10	143.96
		Winter	-1.42	-1.15	2.81	34.18	31.36	587.00	813.87

Table 4.3: Results of benthic chamber metabolism experiments, with results weighted by patch type to give estimates of overall reach-scale metabolism. Daily (24 hour) GPP, ER and NEM are calculated based on the oxygen measurements. The O_2, and CH_4 rates are measures of production, i.e. negative numbers indicate net consumption of the gas during the incubation. As all chamber results from each river and season are used together to calculate each value, standard errors cannot be calculated.

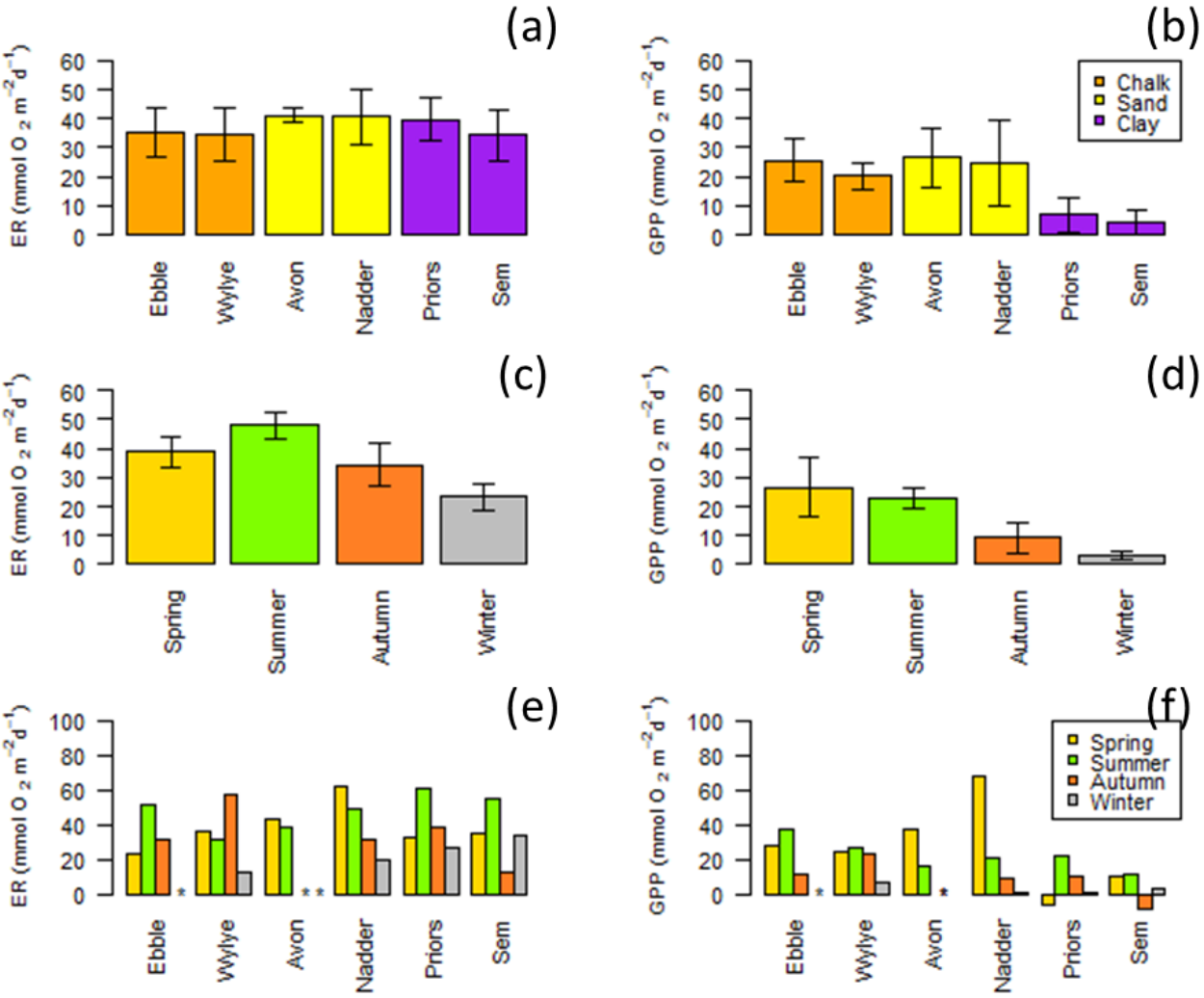


Figure 4.7: (a and c) Respiration (ER) and **(b and d)** gross primary production (GPP) for the sites and seasons. Results are calculated from the chamber flux measurements of O_2, and weighted using the mapping data. Error bars show ±S.E, *n=4* in the top two graphs (a and c); *n=6* in the lower two graphs (b and d). Figures **d and e** show ER and GPP, respectively, each season for each river. No error bars are shown as these figures are based on a single calculation for each river for each season. Two of the GPP calculations resulted in slightly negative results; these would be expected to be positive, but very low and result from a small number of anomalously high measurements of respiration in light chambers.

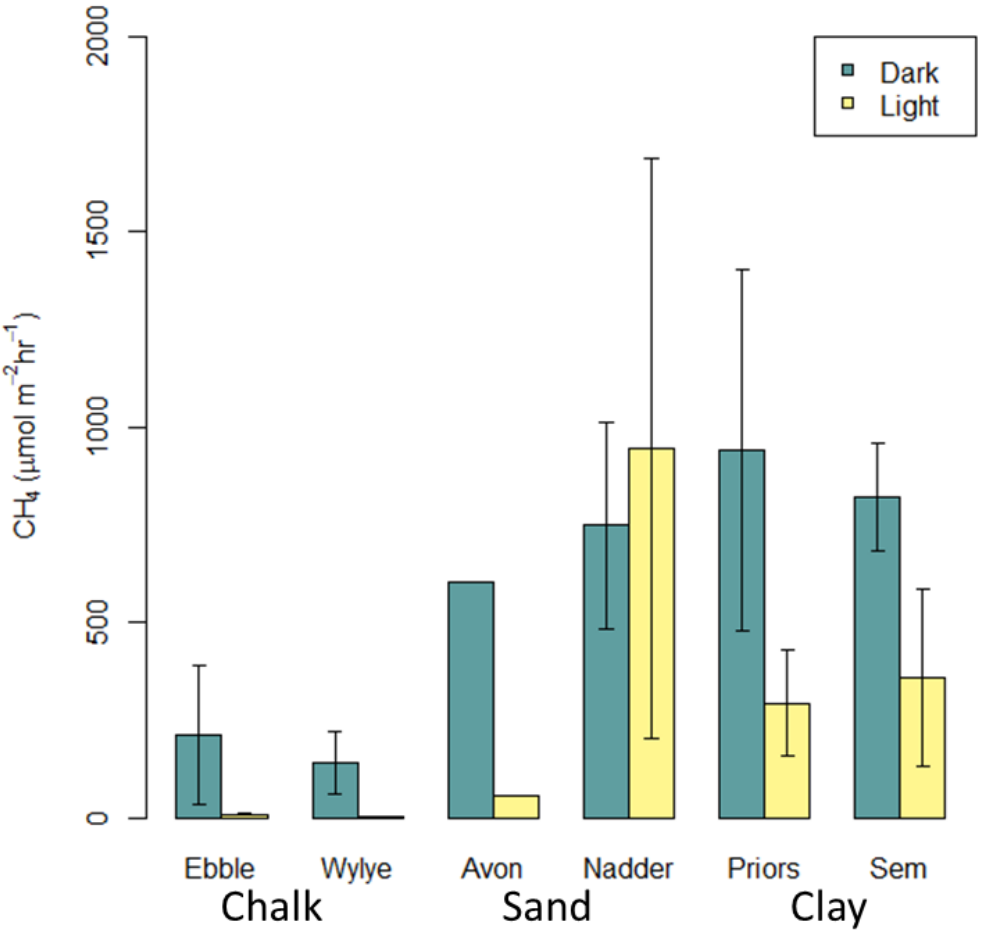


Figure 4.8: Production of methane in each river, comparison between dark and light benthic chambers. Values are weighed by patch type, giving one estimate each river each season, summer, autumn and winter (*n=3*). Error bars show ±S.E. Measurement of methane flux in the Avon are only available in the summer, as such no error bars are given.

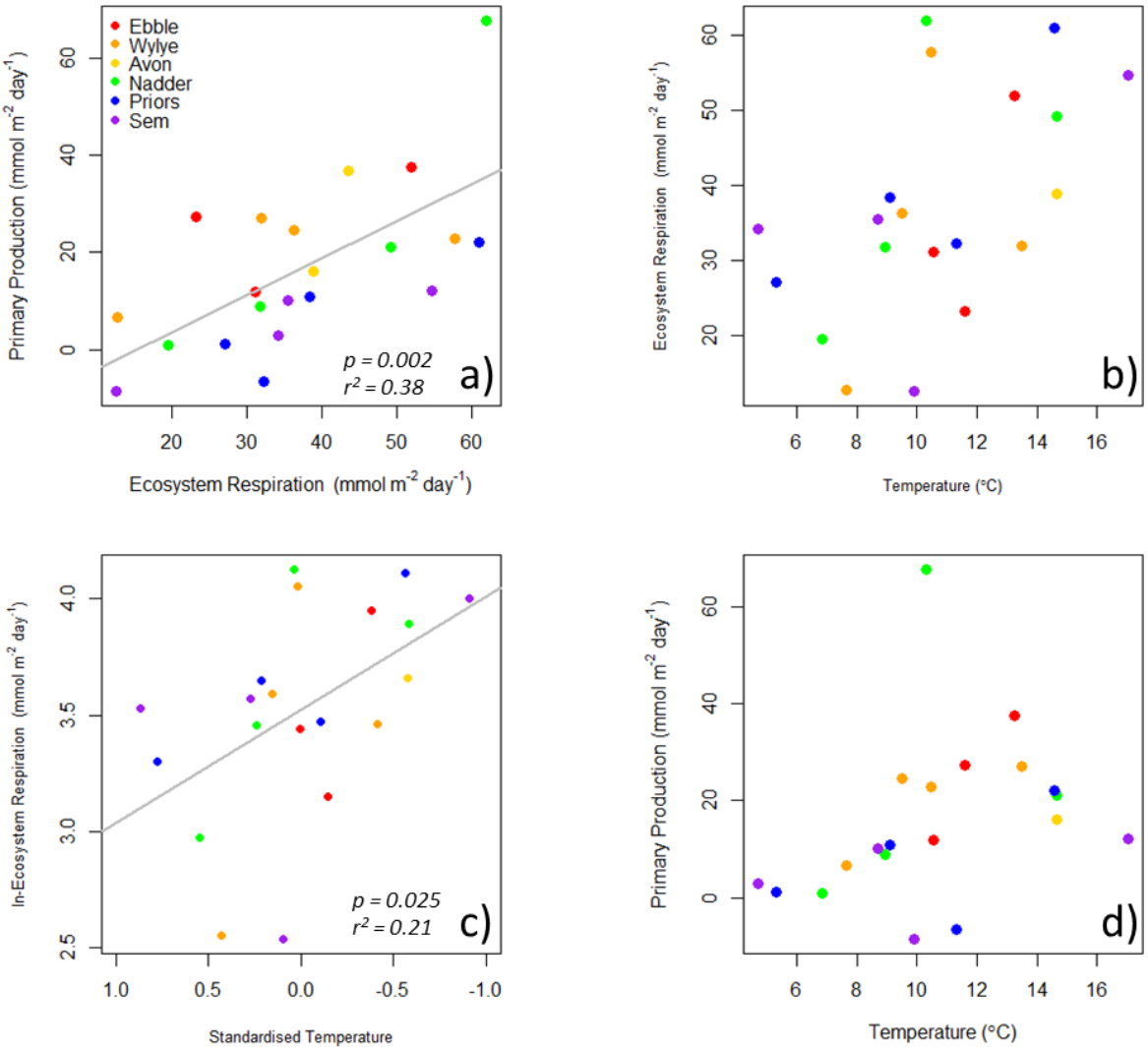


Figure 4.9: Reach scale (patch weighted) metabolism. Relationship between **(a)** ecosystem respiration and primary production; **(b)** temperature and ecosystem respiration, **(c)** standardised temperature and log-transformed ecosystem respiration, and **(d)** temperature and primary production.

4.4.1 Underlying geology determines physical characteristics of riverbeds

The mapping results summarised in Figure 4.3 confirm the difference in substrate in the bed sediments between the different underlying geologies, as also described by the particle size results in Chapter 3. Also confirmed by the mapping is the heterogeneity and presence of different patches in the sand and chalk rivers. Many studies of benthic metabolism will, for simplicity, measure only one type of sediment(e.g. Rees *et al.* 2005; Hedin 1990, Table 4.4). Rees et al include three streams, but do not consider underlying geology or describe differences in sediment; and Hedin uses multiple streams with the same characteristics. However, the results shown here prove that the proportion of coverage of different patches can be large, and that metabolism can vary significantly between them. As such, measuring only one patch can miss the variation in metabolism shown here, and so may cause errors when using these data for scaling up. Integrative measurements such as eddy correlation or O_2 monitoring stations will incorporate the whole reach, however they cannot differentiate the variations in metabolism between patches or areas of the reach. The experiments described here were carried out in order to address this gap, measuring metabolism in different patches then using the mapping carried out to calculate measurements of reach scale metabolism, and explain how much each patch contributes.

One feature the mapping results show is that chalk rivers have significant vegetation coverage, which varies seasonally. This is comparable to what is known about vegetation in chalk rivers, which can cover up to 80% of the riverbed and peak during late spring or early summer(Cotton *et al.* 2006). Base flow index (indicative of the proportion of water derived from groundwater, BFI) is related to vegetation coverage: the chalk rivers, with the highest BFIs, have the highest percentage vegetation cover due in part to their stable flow rates and

clear water.The clay rivers with low BFIs have little or no vegetation, and the sand sites are intermediate. The difference in vegetation coverage, in these study rivers, is important as it is known that presence of vegetation causes retention of fine, nutrient-rich sediment underneath it (Sand-Jensen 1998); which, in turn, can have a measurable effect on biogeochemical cycling (Trimmer *et al.* 2009), including CO_2 and CH_4 production.Thus, it is important to incorporate this in measurements of metabolism, and the seasonal variation.

Base flow index also proved to be a reasonable indicator of light intensity and temperature variance. All rivers recorded a similar average yearly water temperature of around 11˚C. However, as the proportion of groundwater input increases, temperature becomes more stable across seasons. Conversely, the river with the lowest BFI (Sem, (clay)) had the largest variation in temperature. Light intensity at the riverbed was highest in the chalk rivers, due to their clear water and lower riparian vegetation, and decreased with BFI. Both light and temperature were shown here to have a significant effect of carbon metabolism in the sediment. Thus the geology of the river can be a useful indicator ofexpected carbon metabolism.

4.4.2 GPP is dependent on differences in season and river, ER is less variable

In the measurements described here ER generally exceeded GPP, holding with the concept that net heterotrophy is common in rivers and streams, shown in many previous studies(e.g. Acuña *et al.* 2004; Battin *et al.* 2009). As described in previous chapters, this is sustainable in freshwater ecosystems due to the input of allochthonous organic matter from surrounding terrestrial systems. GPP exceeded ER in only two out of six rivers (Ebble (chalk) and Nadder (sand)) and in only one season (spring). This net difference was caused by the variation in GPP seasonally, rather than changes in ER.

Reach-scale rates of GPP varied seasonally, and were highest in spring and lowest in winter. Although this study measured metabolism only in the sediment, this peak in spring was associated with the highest coverage of macrophytes in the chalk and sand rivers. The un-weighted, light chamber measurements of O_2 consumption showed the same patterns as calculated reach-scale GPP; measuring, on average, net production of O_2 during spring and the highest rate of consumption in winter. This was unsurprising and correlated with higher measured light intensity at the riverbed. Other studies of carbon metabolism in riverbeds have also shown seasonal variation in GPP (e.g. Cotner *et al*. 2006), and it is highest in low flow conditions (Roach *et al.* 2014) such as were found in this study during the spring and summer campaigns.

River was a significant factor in net O_2 consumption in the light chambers, with the lowest rate in the Ebble (chalk, highest BFI) and the highest rate in the Sem (clay, lowest BFI) (Table 4.2). However, when these results are used to calculate GPP at the reach scale, rates were consistent across both the chalk and sand rivers, but lower in both clay rivers (Table 4.3, Figure 4.7). As with season, this shows light intensity at the riverbed is a key factor in GPP. Consistently low light intensity was measured in the clay rivers, whilst there were higher rates in both the sand and chalk rivers, which varied seasonally. Although patterns did appear between different geology types (chalk, sand and clay) for estimates of metabolism, linear relationships with BFI were not seen, for the O_2 or CH_4 data. Instead, GPP rates were similar across both chalk and sand rivers, with their clearer waters and lack of shading from riparian vegetation; whilst CH_4 fluxes were common to sand and clay rivers, with their finer sediment particle sizes compared to chalk.

Reach scale estimates of ER were highest in summer and lowest in winter. Winter respiration was, on average, approximately half that measured in summer (Figure 4.7c). All

rivers had the highest reach-scale respiration in summer or spring, except the Wylye where it was highest in autumn. This could be because of the larger proportion of fine sediment, shown by the mapping data, which was present in autumn and originated from the dieback of the submerged vegetation, which covered a higher proportion of the Wylye than other rivers. ER has been shown previously to be positively correlated to benthic organic matter (Bernot *et al.* 2010); as well shown here in the potential measurements in Chapter 3.

Rates of ER were not affected by geology or BFI. This was true at both the chamber scale, where river was an insignificant predictor of ER, and at the reach scale (Figure 4.7a). This is perhaps surprising: rivers with higher local primary production may be expected to have higher rates of respiration compared to clay rivers, particularly in the summer, if autochthonous production was the predominant source of carbon for respiration. On the other hand, in winter the clay rivers, which receive greater amounts of allochthonous carbon from surface runoff during rainfall events, as well as leaves dropped from deciduous riparian trees, may be expected to have higher rates of respiration. Houser *et al.*(2005) found respiration rates in similar streams to be highest in winter, and attributed this to the input of labile organic matter from surrounding terrestrial vegetation. Although ER was not significantly correlated with river, it was positively correlated with GPP (Figure 4.9a). This suggests that supply of autochthonous carbon may be a significant factor in benthic respiration, although it is not the only limiting factor. Reach scale respiration being highest in summer also supports autochthonous production as an important predictor of ER. However, the relationship may not be directly causal; the chalk and sand rivers with high GPP in the sediment also had high coverage of macrophytes, which was higher in summer. Therefore the trapping of fine sediments high in organic matter (discussed in Chapter 3) may at least partly be the cause of the higher rates of ER seen here.Despite the pervasiveness of allochthonous carbon in freshwaters, autochthonous carbon is considered a preferable source for respiration, due to its

high nutritional quality (Roach 2013). Nonetheless, the nearly constant net heterotrophy measured across rivers and seasons, together with the lack of BFI influence on ER, suggests that at least a portion of the carbon metabolised by the benthic microbial community must be allochthonous, in all rivers across the geological gradient.

Rates of reach scale ER were positively correlated with water temperature. As shown in Figure 4.9c, metabolism in all rivers responded to changes in temperature. It is widely known that metabolic rates increase exponentially with temperature (Brown *et al.* 2004), and these findings are in agreement with the potential measurements described in Chapter 3 as well as other studies in the literature (Yvon-Durocher *et al.* 2012) Yvon-Durocher et al carried out a meta-analysis of a range of ecosystems including freshwater, oceanic terrestrial systems to show a consistent response of ecosystem respiration to seasonal changes in temperature. The activation energy calculated here (0.49 eV) is virtually the same as the average activation energy calculated for the potential measurement results in Chapter 3 (0.50 eV). However it is lower than the theoretical, calculated value for respiratory reactions of 0.65 eV (Gillooly *et al.* 2001); indicating other limiting factors affecting the rate of respiration. Additionally, the relationship between temperature and respiration has a relatively low r^2 of 0.21, indicating large variation between rivers and seasons. Therefore, although temperature does contribute to rates of respiration in these river sediments, there are other contributing factors, such as substrate availability, which reduce the response to temperature overall, and cause variability between rivers and seasons. Furthermore, temperature is more variable in the clay sites and more stable in the chalk sites (Figure 4.4). Considering this, together with the differences in GPP between rivers described above, it could be the case that the controls on respiration in different geologies are different; with temperature more important in clay rivers whilst primary production and seasonal patterns have more of an effect in chalk and sand rivers.

The patch measurements show that there are some differences in oxygen metabolism at this scale, with more respiration measured in the sand, fine sediments and vegetated patches compared to gravel in the chalk sites; and more respiration in the vegetated patches compared to the main sand bed in the sand sites. This proves the value of doing measurements of all patches, together with mapping reach coverage, in order to accurately estimate carbon metabolism at larger scales. The differences between main channel and fine sediments are comparable with other chamber studies *in situ* (Bunn *et al.* 2003)(Table 4.4); however when compared to the potential measurements of CO_2 production described in Chapter 3, the differences are much slighter here. This is an important reminder of the limitations of laboratory-based measurements, which do not include all the biological and physical variations in a riverbed, which can affect for example oxygen concentration and hyporheic exchange. These factors may limit metabolism in natural environments in ways that do not occur under the controlled conditions of laboratory analysis.

Study	Method	Location	Respiration ($g\ O_2\ m^{-2}\ d^{-1}$)	GPP ($g\ O_2\ m^{-2}\ d^{-1}$)	Notes
This study	Chambers	SW England	Spring: 1.24 ±0.17 Summer: 1.53 ±0.14 Autumn: 1.10 ±0.23 Winter: 0.75 ±0.15	Spring: 0.85 ±0.33 Summer: 0.73 ±0.12 Autumn: 0.29 ±0.16 Winter: 0.09 ±0.04	Streams on a gradient of geologies.
Bunn et al. 2003	Chambers	Lake Eyre, Australia	Littoral: 0.27 - 6.56 Channel: 0.06 - 0.33	Littoral: 0.18 - 10.71 Channel: 0.03 - 0.23	Waterholes on a floodplain
Rees et al. 2005	Chambers	SE Australia	0.127 - 2.178	NA	Lowland rivers – range of from 3 sites.
Houser et al. 2005	Single station	Georgia, USA	Winter: 1.3 - 16.3 Spring: 0.8 - 10.7 Summer:0.2 - 5.2 Autumn: 0.1 - 3.3	Winter: <0.01 - 0.92 Spring: <0.01 - 1.75 Summer:<0.01 - 0.29 Autumn:<0.01 - 0.44	Measured against a gradient of disturbance intensity.
Hedin 1990	Chambers	New Hampshire, USA	0.07-0.91	NA	Shaded woodland streams
Acuña et al. 2004	Single station	Spain	0.4 – 32	0.05 - 1.9	Third order stream
Uehlinger et al. 2002	Single station	Arizona, USA	1.65 ±0.13	0.3 ±0.1	Desert stream

Table 4.4: Comparison of O_2 metabolism in this study with other published studies. Results are expressed in grams of O_2, as this was the unit used for the majority of these studies. However, results throughout this chapter are expressed in moles due to the equivalence in respiration between moles of O_2 consumed and moles of CO_2 produced.

4.4.3 Methane production is higher in the dark, and in vegetated sediment

Methane production was measured in all rivers but there were differences in the magnitude across patches, rivers and between light and dark chambers. It is important to note that these experiments measured the net flux of CH_4 between the sediment and the overlying water, i.e. the sum of methanogenesis and methanotrophy. Most of the incubations resulted in an increase in CH_4, therefore there was higher methanogenesis than methanotrophy. However, this is not the total rate of methanogenesis occurring. In lakes, 30-99% of methane produced is oxidised by methanotrophic bacteria (Bastviken *et al.* 2008), predominantly under aerobic conditions in the top few millimetres of sediment.

Measurements were characterised by severe heterogeneity, with fluxes spanning three orders of magnitude, even within the same river and deployment. These findings are in common with other studies of methane production (Adrian *et al.* 1994; Wachinger *et al.* 2000); demonstrating that methanogenesis occurs in 'hot spots': areas of anoxia in which conditions are preferable for methanogens. Where alternative election acceptors, such as sulphate or nitrate are present, methanogens are out-competed for H_2 and acetate (Achtnich *et al.* 1995; Borrel *et al.* 2011) by denitrifying and sulphate or iron reducing microorganisms.

Unlike CO_2, which is readilydissolved in water, CH_4 is largely insoluble and so significant amounts can be released in bubbles as ebullitive flux. Large bubbles, released sporadically, can be a cause of the heterogeneity of measurements. As the CH_4 fluxes measured here were estimated from two point measurements, at the start and end of the incubations, it is not possible to calculate how much of the flux may have come from ebullition. Ebullition can be increased by disturbance of the sediment. However, this is unlikely to have increased total flux in these measurements as after installation of the bottom of the chambers, they were left for 30 minutes to settle before adding the rest of the chamber(See Section 4.2.2).

In this study, neither season nor temperature were found to be significant predictors of methane flux from riverbeds. This is in contrast to previous studies (Duan *et al.* 2005; Wang & Han 2005), which have found CH_4 emissions from lakes and marshes are positively correlated with temperature. This may be because increased methanogenesis can cause an increase in methane oxidation by methanotrophs in response (Shelley *et al.* 2015); although there is greater methane production, there is no net increase in flux from the sediment due to increased methanotrophy. There was, however, a correlation between net methane flux and net ecosystem metabolism, suggesting availability of substrate on the local scale is a more important limiting factor on methanogenesis in these. Net ecosystem production has been correlated with CH_4 emissions across a range of ecosystems (Whiting & Chanton 1993), who estimate that 3% of daily NEP is emitted to the atmosphere as CH_4.

Differences in methane flux between rivers were found in this study. The two chalk rivers (Ebble and Wylye) had significantly lower rates of methane flux than the clay and sand rivers. Low rates of net methane production in the gravels reflects the lower organic matter and higher oxygen concentration due to mixing of overlying water, inhibiting methanogens. Anoxic, marginal fine sediments in streams and rivers have been identified as sites of methane production (Bonnett *et al.* 2013; Shelley *et al.* 2015).

When individual patch metabolism was investigated, significant rates of net methane production were found in the vegetated areas of the chalk riverbeds, comparable to those in the sand and clay. This is likely due to the fine, nutrient-rich sediments present in these areas (Sanders *et al.* 2007), as reported in previous studies (Clarke 2002; Cotton *et al.* 2006) as well as in the measurements of organic carbon, nitrogen and chlorophyll described in Chapter 3. The higher rates of methane production in fine sediments are also consistent with findings from Chapter 3, which demonstrated an inverse correlation with particle size (principal component 2). These findings validate again the importance of patch considerations when measuring rates of carbon metabolism in sediments.

Regarding differences in carbon metabolism between patch types in freshwaters, previous studies have found an effect on both CO_2(Bunn *et al.* 2003) and CH_4(Shelley *et al*. 2014) production. The advantage to this study is that as well as measuring metabolism in the different patches, the mapping of the riverbeds carried out allowed these patch-scale measurements to be scaled up to whole reach estimates.

In the Nadder (a sand river); dark chamber flux measurements of 498 µmol $CH_4m^{-2}hr^{-1}$ were measured in the main sand, whilst measurements in the vegetated patches recorded an average of 988 µmol $CH_4m^{-2}hr^{-1}$. This represents an increase of 98% in methane emissions under vegetation, which covers between 11-16% of the riverbed throughout the year. Thus, only measuring fluxes from the sand would underestimate the amount of CH_4 released from sediment in this river significantly, by 107 µmol $CH_4m^{-2}hr^{-1}$ on average throughout the year. Differences are even more pronounced in the Wylye, where only negligible rates of methane production were measured in the gravel, but fluxes of 239 µmol CH_4 m^{-2} hr^{-1} were measured in the dark in vegetated areas, which cover up to half the river bed in summer. In this case, the river would go from a borderline sink to a reasonable sized source of CH_4 when vegetated areas are considered. Methane emissions from freshwaters account for an estimated 103 Tg yr^{-1} globally (Bastviken *et al.* 2011), but there are known discrepancies between these estimates, derived from process-based 'bottom up' estimates, and 'top down' estimates using atmospheric observations (Kirschke *et al.* 2013). Properly considering differences in patches, as well as other factors which contribute to the heterogeneity seen in CH_4 fluxes, could be one way in which to improve these estimates.

These experiments have shown a difference in net methane between the dark and light chambers: approximately twice as much methane was produced in the dark. Where previous studies have also considered light or diel effects on methane fluxes, there have been conflicting reports of the difference between light and dark. Currently there is not a clear understanding of the relationship between methanotrophy or methanogenesis and light.

Since the methane fluxes measured here are a combination of both production by methanogenic archaea and oxidation by methanotrophic bacteria, the difference between light and dark may be due to changes in the rate of one or the other or both. A study of methanotrophy in a reservoir (Dumestre *et al.* 1999) and another in lake water (Murase & Sugimoto 2005) found that net emissions are lower in the dark and concluded that this was due to inhibition of methanotrophy by light. In contrast, King (1990) found that exposing sediment cores from wetlands to light caused a reduction in methane production, relative to the same sediment cores in the dark. Additionally, samples with algal mats in that study were more susceptible to changes in light than those without. In this case it was suggested that increased oxygen availability due to photosynthesis in the light allowed a higher proportion of methane to be oxidised by methanotrophs. The results presented in this study support this hypothesis that there is higher net production of methane in the dark than in light. This may be due to the increased availability of O_2 under light conditions. However, if that were the case it may be expected that the clay sites would have a smaller difference between light and dark, due to the very low rates of GPP described above. With the very heterogeneous results and the comparatively small sample sizes, it is difficult to test this; though it does appear from the data available that the chalk rivers may have a greater difference between dark and light chambers than the clay. Further research is needed in this area; and potential differences in dark and light fluxes should be considered when scaling up measurements of sediment carbon metabolism.

4.5 Conclusion

These experiments are unique in describing metabolism of both O_2 and CH_4 across such a range of riverine ecosystems, both in terms of inter and intra-reach variation. They also incorporate both light and dark conditions, and seasonal effects.

Season and geology were found to be important for GPP, whilst temperature was the most important variable to explain ER. Intra-reach variation and the heterogeneity of river beds was found to be a significant cause of variation in net CO_2 and CH_4 production, especially in the chalk rivers. Overall, strong heterotrophy as well as positive fluxes of methane were found across the rivers and seasons, reaffirming the notion of rivers and streams as being net sources of these greenhouse gases to the atmosphere.

The finding of higher methane fluxes in dark conditions compared to light is novel under these conditions, and should be further investigated.

There are some differences between the findings in this Chapter and the previous, which will be explored in more detail in Chapter 6.

This Chapter has improved knowledge about spatial and temporal variability in carbon metabolism in rivers in temperate conditions. However, the overall flux of CO_2 and CH_4 to the atmosphere is dependent not only on this, but interactions with physical and biological factors in the overlying water and terrestrial feedbacks. This will be the focus of the next Chapter.

4.6 References

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5. Hydrological and biological control on CO_2 and CH_4 emissions from rivers to the atmosphere

5.1 Introduction

5.1.1 Background

Freshwaters, particularly headwater streams, are usually supersaturated in carbon dioxide (CO_2) and methane (CH_4) (e.g. Aufdenkampe *et al*. 2011; Hope *et al*. 1994; Crawford *et al.* 2013), and hence are a source of these greenhouse gases to the atmosphere. Previous chapters have discussed the role of benthic metabolism as a source of CO_2 and CH_4 to the water column, and demonstrated how this is affected by differences in underlying geology, as well as variations due to light and seasonal changes.

However, the magnitude of CO_2 and CH_4 out-gassed from the stream to the atmosphere may not be entirely dependent on local metabolism. The hydrological connection between the surrounding terrestrial catchment and receiving water provides a supply of dissolved gases from groundwater and surface water runoff in addition to benthic production (Cole & Caraco 2001). The contribution of benthic metabolism to total out-gassing to the atmosphere is not well quantified in overall riverine carbon budgets (Striegl *et al.* 2012). Still, one recent analysis used river and stream data in the USA to estimate that internal CO_2 production contributes only ~28% to total out-gassing, with the rest primarily being from terrestrial input (Hotchkiss *et al.* 2015).

The effects of geology may be hypothesised to affect the lateral input of gases from the catchment, as well as affecting benthic metabolism. Underlying geology affects the lateral transport of water and dissolved compounds from the terrestrial catchment as has been discussed in previous chapters, as well as affecting the proportion of stream water which comes from rainfall runoff, compared to groundwater input. Thus far this thesis has discussed how underlying geology can affect availability of organic matter and thus benthic metabolism,

but CO_2 and CH_4 derived from weathering and terrestrial catchment metabolic processes can also be delivered to the river or stream (Hope *et al.* 2004), and be out-gassed without in-stream microbial processing. Seasonal cycles may also influence out-gassing of CO_2 and CH_4. Seasonal changes in rainfall and storm events alter the amount and the pathways by which water and dissolved gases are delivered to the river channel, and consequently may influence the concentration of dissolved gases in river water.

Studies typically estimate river and stream CO_2 out-gassing using CO_2 concentration measurements in the water; but direct measurements of gas flux to the atmosphere are limited. Estimated flux measurements using CO_2 concentration include inherent uncertainty due to the requirement to estimate the gas transfer velocity, *k*. This is affected by many variables such as wind speed, bubbles and water-side convection, which are not always known (Podgrajsek*et al*. 2014). In addition, few studies of CO_2 out-gassing incorporate diel or seasonal cycles. Where they have been measured, seasonal and diel changes in CO_2 can be significant (Lynch *et al.* 2010; Peter *et al.* 2014); in particular measuring only daytime flux can underestimate CO_2 emissions significantly (Peter *et al.* 2014). Fluxes of CH_4 are seldom included, and consequently are less well understood (Crawford *et al.* 2013). However, CH_4 comprises a significant proportion of the total carbon budget in freshwaters (Cole *et al.* 2007), and is a much more potent greenhouse gas than CO_2. In lakes, when expressed as CO_2 equivalents, CH_4 emissions can be larger than CO_2, making CH_4 the dominant greenhouse gas (Schrier-Uijl *et al.* 2010).

Using floating chambers, it is possible to measure directly the net exchange of gases between the stream water surface and the atmosphere, without needing to estimate a gas transfer velocity. When carried out concurrently with measurements of benthic metabolism, the proportion of CO_2 and CH_4 emissions attributed to local metabolism can be directly calculated. The focus of this chapter will be to present flux data generated using floating chambers and discuss how this compares to the benthic metabolism data amassed in the

previous chapter, as well as the effects of variations in catchment geology, diel and seasonal cycles.

5.1.2 Outline of Chapter

Whilst the previous two chapters were concerned with local metabolism within the riverbed, this chapter describes experiments carried out to establish the extent of CO_2 and CH_4 out-gassing from the rivers to the atmosphere. The purpose initially was to find the magnitude of out-gassing as well as variations across the geology types and seasons, as well as diel changes.

This data is then combined with the metabolism measurements from the previous chapter, in order to calculate what proportion of out-gassing can be explained by in situ processes. Additional, hydrological data from the sites is used to determine other sources of CO_2 and CH_4 and explain how these are better indicators of riverine out-gassing. Again, these analyses will be carried in the context of variable geology type.

5.1.3 Objectives

- To measure the amount of CO_2 and CH_4 emitted from the six study rivers, with contrasting geology, during the sampling campaign of Chapter 4.
- To obtain high frequency, day and night (diel) measurements of CO_2 and CH_4 emissions from the study sites.
- To establish how emissions vary according to seasonal changes, including changes in rainfall.
- To establish what proportion of CO_2 and CH_4 out-gassed by rivers can be explained by local metabolism,by comparing these results with those in Chapter 4.

5.2.1 Sampling campaigns

There were two major sampling campaigns to measure out-gassing of CO_2 and CH_4. The first was run concurrently with the seasonal sampling campaign described in chapter 4. Out-gassing of CO_2 and CH_4 was measured during the day at the same time as the benthic chamber incubations were being carried out. Between 1 and 3 measurements were taken per river, per season.

The second intensive campaign was carried out during spring 2015. Experiments were carried out at each of the six rivers described in Chapter 4 (Ebble, Wylye, Avon, Nadder, Priors and Sem); for 3 days and 2 nights. The aim here was to get high frequency measurements of out-gassing of both CO_2 and CH_4, and to include the full 24 hour diel cycle.

5.2.2 Experimental design

The floating chambers used during the seasonal campaigns (Figure 5.1) are made of inverted plastic bowls covered in reflective aluminium tape, and were supplied by David Bastviken. They contained a sensor which measured and recorded CO_2concentration by infra-red spectroscopy (SenseAir; Delsbo,Sweden, precision as coefficient of variation measured as better than 5%) connected to a 9V battery, and also had a polyurethane tubing sample port connected to allow manual gas samples to be taken via a three-way luer-lock valve. The chambers enclosed a volume of 8.6L and covered a surface area of 674cm^2.They were installed by floating them on the surface of the water in the centre of the river, in the main channel. They were fixed by attaching a weight (a brick) using nylon cord,and placing it on the river bed, and by attaching the chamber using more nylon cordto a metal post on the river bank. Using fixed chambers can cause artificially high rates of outgassing when compared to drifting chambers due to increased turbulence (Lorke *et al*. 2015). This effect should be minimal in the

rivers used in the study, however, as effects are most pronounced in rivers with much higher flow rates than those experienced in the study sites; even at periods of high discharge. Another issue that can cause inaccuracies with floating chamber measurements is that, within the closed chamber, gases can reach equilibrium with the underlying water and so the rate of out-gassing is affected. This was reduced here by keeping the amount of time the chambers were closed for to the minimum needed to collect the data and excluding data if the rate became non-linear.

The chambers were deployed in the morning, for approximately 30 minutes, with the CO_2 sensors set to measure the air every 5 minutes. During the same time period, samples were taken from the sample port at regular intervals using a gas-tight syringe (SGE International Pty Ltd, Australia). The gas was then stored for later analysis by displacing degassed deionised water from 3mL gas tight vials (Exetainers, Labco, UK).

For the spring 2016 intensive campaign, a different floating chamber system was used (Figure 52). The chamber used was a Li-Cor Long-Term Chamber 8100-101 (Li-Cor, Nebraska, USA) (volume: 4093 cm^3, surface area covered: 318 cm^2)...They were modified to be used over water by the addition of a plastic cylinder and life ring. These were also anchored to the river bed using bricks attached by nylon string.

The chamber was connected to a CR800-Series Datalogger (Cambell Scientific Inc, Utah, USA) which was used to control when the chamber opened and closed. It was set to alternately be open for 10 minutes, then closed for 10 minutes. This meant during the open periods the chamber was flushed, so the concentration of gases in the chamber did not reach equilibrium with the underlying water. The chamber was also connected to an Ultraportable Greenhouse Gas Analyzer (Los Gatos Research, California, USA). This took high frequency measurements of the concentration of CO_2 and $CH_4$by cavity ring spectroscopy approximately once every 10 seconds. It also measured water vapour, allowing results to be corrected for water vapour dilution.It was attached to the chamber by a closed loop so the rate of flux from

the river to the air could be measured. The precision as a coefficient of variation was found to be better than 1% for both CO_2 and CH_4.

The plastic tubes carrying the air between the chamber and the Greenhouse Gas Analyser, and the data cable connected the chamber to the Datalogger (Figure 5.2a) were bundled together within plastic tubing. The Datalogger and the Greenhouse Gas Analyser were stored together with the battery powering them in a waterproof steel box on the river bank, with a hole in the side to feed the lines through.

As with the seasonal floating chamber method, the chambers were placed in the main channel of the river bed, over areas that were representative of the predominant riverbed sediment.

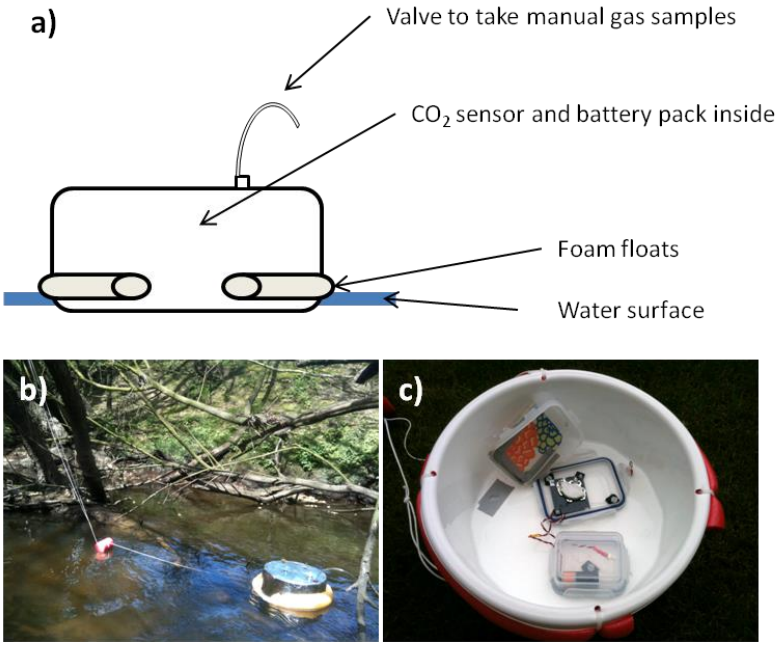

Figure 5.1: The floating chamber used to measure CO_2 and CH_4 flux to the atmosphere during the seasonal campaigns, concurrently with measurements of benthic metabolism described in Chapter 4. **(a)** is a diagram of the chamber; **(b)** shows the chamber whilst deployed on the river Nadder; **(c)** shows the inside of the chamber, with CO_2 sensor and battery pack in plastic containers.

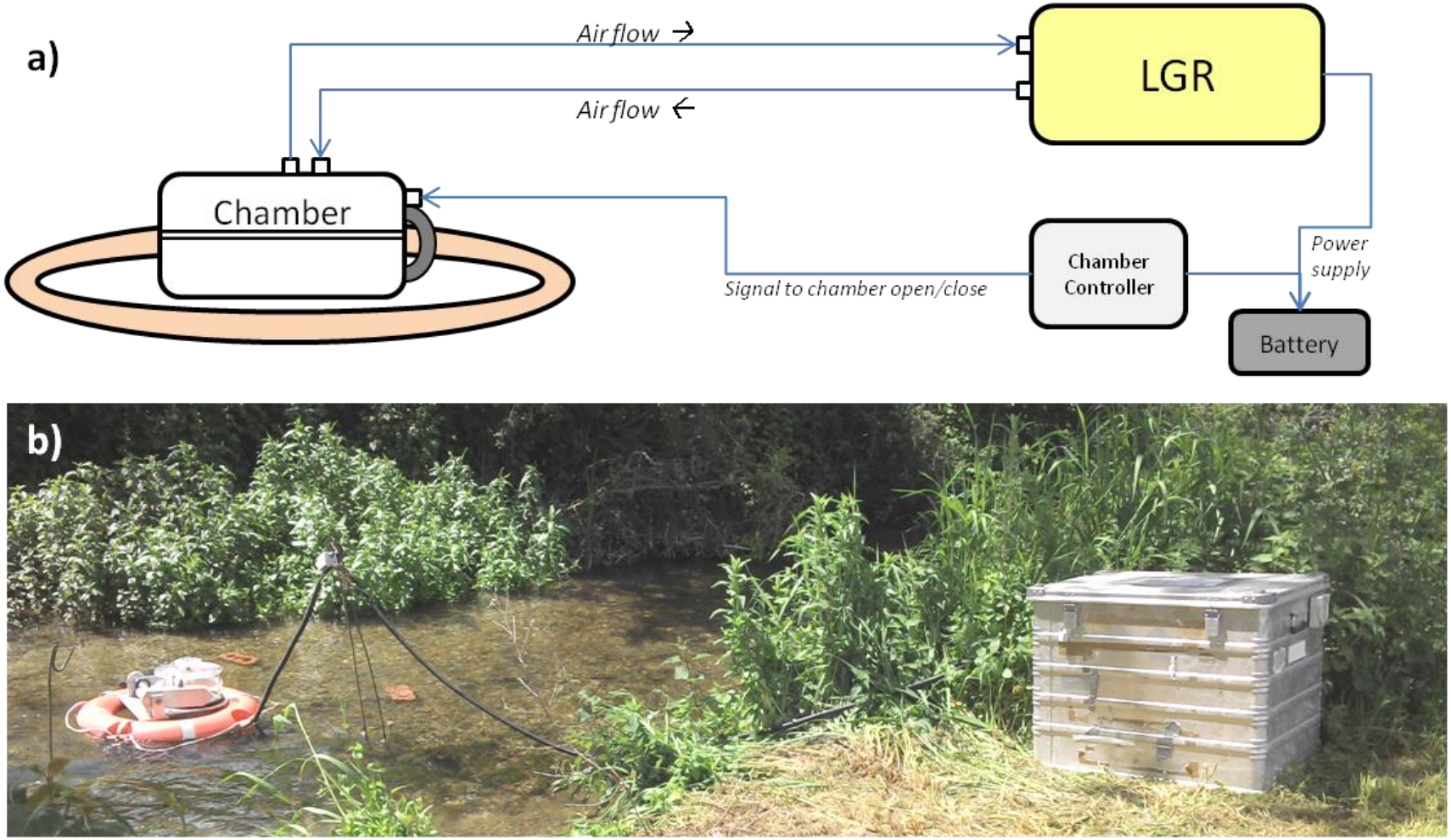

Figure 5.2: The chamber used to measure CO_2 and CH_4 flux to the atmosphere during the spring 2015 high resolution campaign, which included full 24 hour measurements. **(a)** is a diagram of the chamber, connected to the Los Gatos Analyser (LGR), which sampled the air from the chamber to measure CO_2, CH_4 and water vapour and the chamber controller, which opened and closed the chamber at pre-defined intervals to allow flushing of the air. The LGR and chamber were on a closed system, with air returned to the chamber after sampling. **(b)** is a photo of the chamber, deployed in the River Ebble.

5.2.3 Laboratory Analysis

The gas samples generated during the seasonal campaign were analysed for CO_2 and CH_4 using gas chromatography according to the method described in Section 3.2.3.

5.2.4 Data analysis

The results from the timed seasonal campaign samples and CO_2 sensors were converted into rates of flux using linear regression. Only time points during the linear phase were used: if the chamber was left for long enough that the concentrations in the chamber began to equilibrate with the water these data were discarded. The flux and the dimensions of the chamber were then used to calculate the flux of CO_2 and CH_4 in mmol $m^{-2}hr^{-1}$.

The data generated from the spring 2015 campaign were saved by the LGR analyser in text files. A programme was written in R (R Core Team, 2015) to calculate the flux, using linear regression, for each of the 10 minute intervals during which the chamber was closed. Again, the dimensions of the chamber were used to calculate the flux of CO_2 and CH_4 in mmol $m^{-2}hr^{-1}$.

This Chapter also includes data from hydrological monitoring described in Section 2.3. Discharge data was used to compare with CO_2 and CH_4 fluxes.

5.3.1 Intensive sampling

River	Geology	Carbon Dioxide Flux (mmol m^{-2} hr^{-1})				Methane Flux (µmol m^{-2} hr^{-1})			
		Dark	*S.E.*	Light	*S.E.*	Dark	*S.E.*	Light	*S.E.*
Ebble	Chalk	9.88	*0.75*	8.42	*0.44*	20.14	*1.49*	23.68	*1.07*
Wylye		2.51	*0.07*	1.75	*0.06*	2.30	*0.34*	2.48	*0.20*
Avon	Sand	10.22	*0.54*	6.83	*0.26*	44.56	*1.09*	37.43	*0.86*
Nadder		3.71	*0.11*	2.56	*0.11*	47.24	*1.15*	46.57	*0.72*
Priors	Clay	0.43	*0.06*	0.39	*0.02*	1.83	*0.21*	1.63	*0.04*
Sem		1.54	*0.05*	1.17	*0.06*	18.70	*2.92*	18.47	*6.80*

Table 5.1: Summary of carbon dioxide and methane flux results from intensive, three week sampling campaign during Spring 2015. 'Dark' is defined as between sunset and sunrise; 'light' is defined as between sunrise and sunset. Average fluxes are from approximately 72 hours of data for each river, with approximately one flux measurement each 30 minutes.

The results of the intensive, spring 2015 sampling campaign are summarised in Table 5.1. These show average fluxes in the dark and light, defined as between sunrise and sunset. The sand sites (Nadder and Avon) have the highest out-gassing of CH_4, whilst the Wylye (chalk) and Priors and Sem (clay) have low rates of both CO_2 and CH_4 out-gassing.

As Figure 5.3 shows, there were diel trends in CO_2 out-gassing, with highest CO_2 fluxes during the night, reducing from the early hours of the morning and the lowest fluxes between midday and early afternoon. However there were variations between days and rivers; the Ebble (chalk) in particular measuring a lower flux during the second night of deployment than during the days. Also, these trends are strongest in the chalk rivers, lower in the sand rivers and very weak in the clay rivers. This diel signal however was not seen for CH_4 out-gassing.

The relationship between CO_2 and CH_4 flux was plotted individually for each river (Figure 5.4); and show a relationship in some cases. However there are differences between rivers. The Ebble (chalk) and Avon (sand) show the greatest variation in both CO_2 and CH_4 over the

measurement period; whilst the Nadder (sand) shows a lot of variation in CH_4 out-gassing but relatively constant CO_2 out-gassing. The Wylye (chalk) and the two clay sites (Priors and Sem) again show the lowest rates of out-gassing of both CO_2 and CH_4, although the Sem does have a small number of high fluxes of CH_4. Also apparent from Figure 5.4 is that all rivers are overwhelmingly net sources of both CO_2 and CH_4, with only the Ebble showing fluxes below zero (i.e. net movement from the atmosphere into the river) on a handful of occasions.

Next, the relationship between fluxes during the day and night from this dataset were investigated, by comparing all fluxes during daytime (i.e. sunrise to sunset) with all fluxes during night-time (i.e. sunset to sunrise). As shown in Figure 5.5, the proportional difference between light and dark measurements of both CO_2 and CH_4 flux is very similar across all rivers. For CO_2, 1.3 ±0.12(S.E.) times as much CO_2 is emitted during night compared to average daytime fluxes, across all rivers (r^2=0.96, P<0.001). For CH_4, the relationship is also highly conserved across rivers (r^2=0.97, P<0.001), but is not significantly different from a 1:1 light:dark ratio; i.e. there is the same rate of CH_4 out-gassing during the daytime as there is at night.

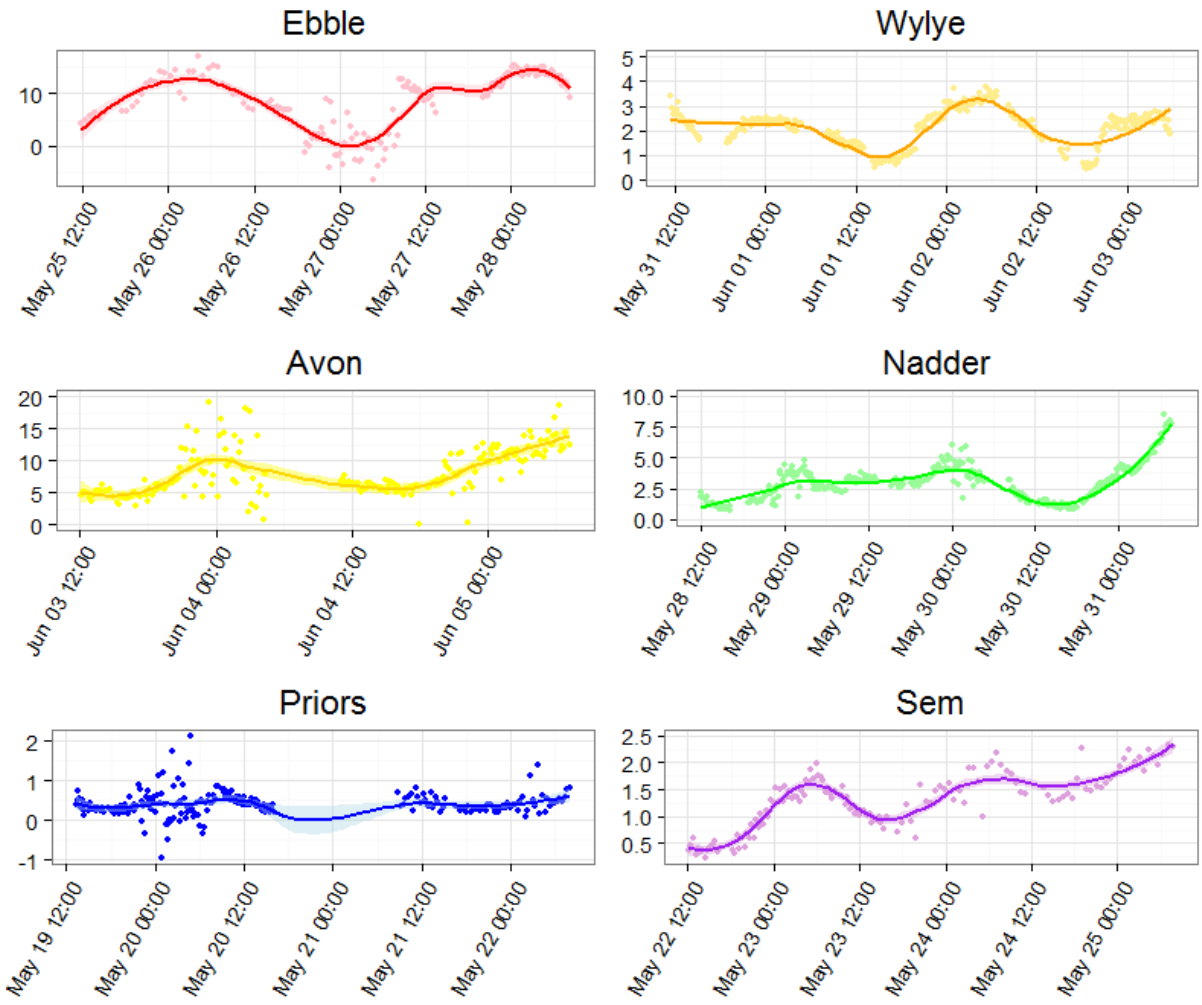

Figure 5.3: Loess (locally weighted regression) smoothed time series of CO_2 out-gassing for each river. Data are from all measurements of flux during intensive sampling campaign of spring 2015; approximately one measurement each 30 minutes. Shaded areas around curve are 95% CI.

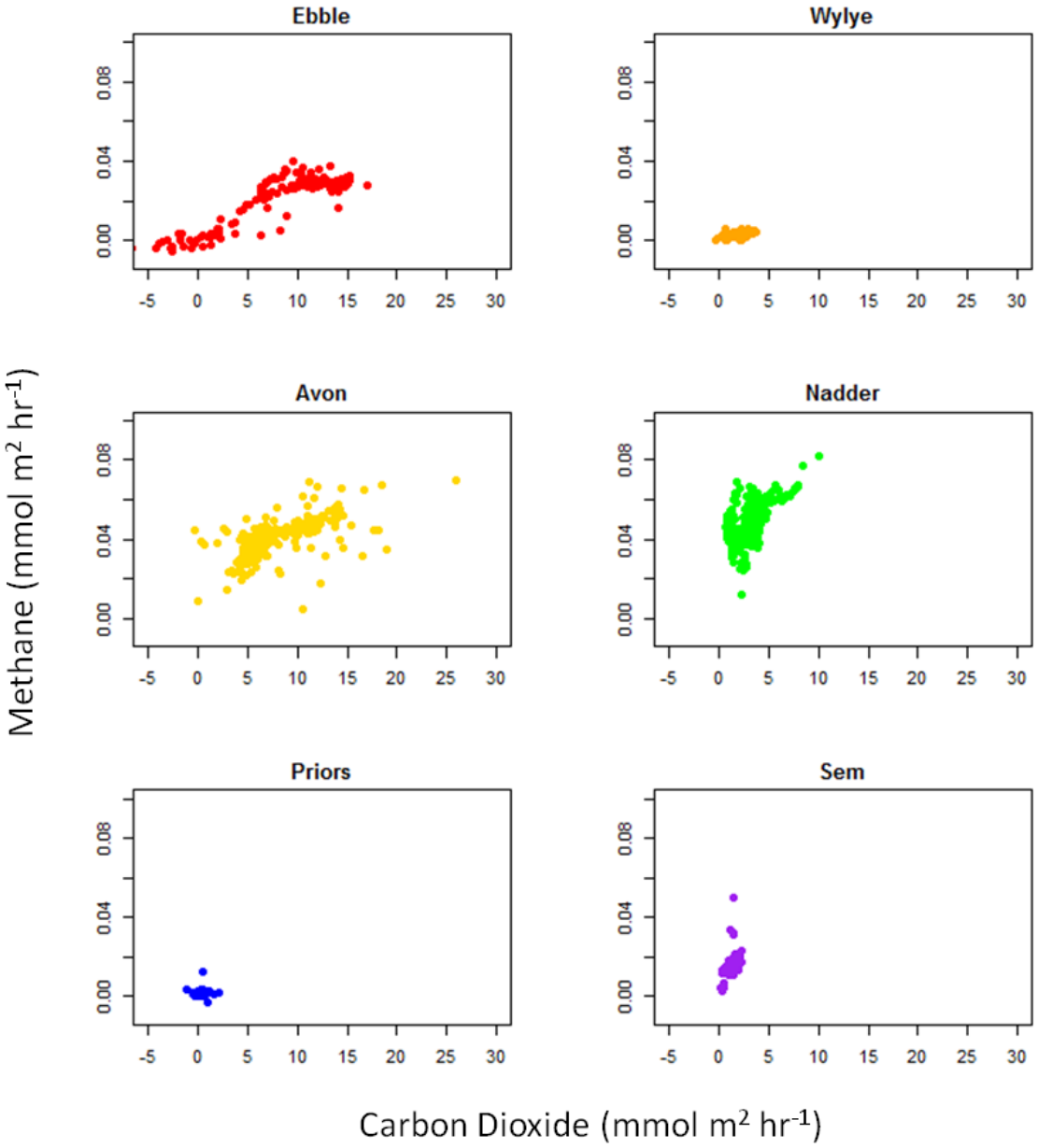

Figure 5.4: Relationship between carbon dioxide and methane flux during intensive, spring 2015 sampling campaign.Rivers are chalk (top row) sand (middle row) and clay (bottom row).

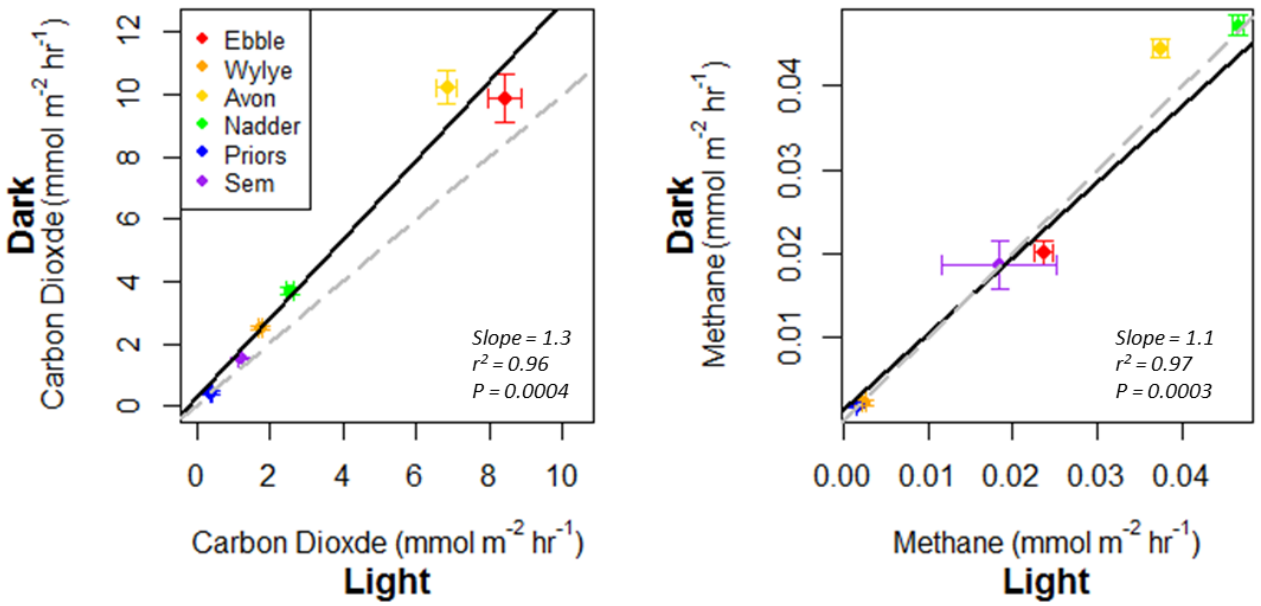

Figure 5.5: Relationship between average day-time (light) and night-time (dark) fluxes from river to atmosphere in **(a)** carbon dioxide and **(b)** methane, for each river. Results are from intensive spring 2015 sampling campaign. Error bars indicate ±S.E. Solid black lines show slope; dashed grey lines show 1:1 relationship.

5.3.2 Seasonal samples

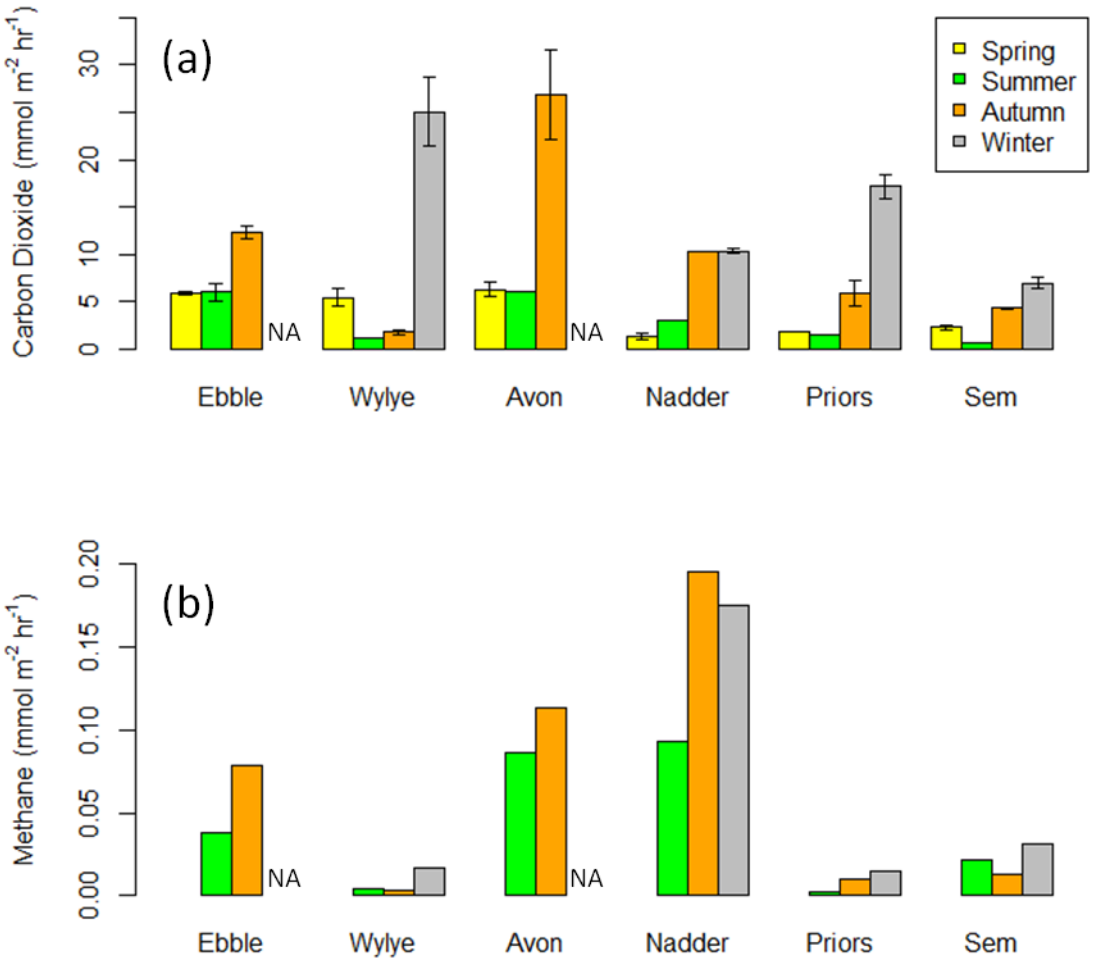

Figure 5.6: Results of floating chamber seasonal study, showing flux of **(a)** carbon dioxide and **(b)** methane from river to atmosphere. Error bars show ±SE. Between 1 and 5 replicate measurements were taken for each river, each season. Where no error bars are shown, only one measurement was taken for that river in that season. It was not possible to take measurements from the Ebble and Avon during winter due to flooding. No measurements for methane were taken for any site during the spring.

The results of the seasonal sampling campaign are summarised in Figure 5.6. These results show fluxes of CO_2 and CH_4 on a similar scale during spring and summer to those obtained during the daytime intensive campaign, described above. However, measurements taken during autumn and winter show a marked increase, for CO_2 in particular (at least double), across all the rivers.

During spring and summer the permeable (chalk and sand) rivers had a higher CO_2 flux than the clay sites. Values range between 0.76 mmol $m^{-2}hr^{-1}$in the Sem (clay) in summer to 6.31 ±0.74 mmol $m^{-2}hr^{-1}$in the Avon (sand) in spring. During autumn and especially winter the flux from all sites markedly increased, with the highest value measured again in the Avon, during autumn of 26.8 ±4.7 mmol $m^{-2}hr^{-1}$. No measurements were taken from the Ebble or Avon during winter due to adverse weather conditions. For the rivers that were measured, all have more than twice as high CO_2 out-gassing during winter compared to summer.

Fewer measurements of CH_4 were taken than CO_2 and no measurements were taken in spring; however the permeable rivers again have higher fluxes than the clay rivers, with the exception of the Wylye (chalk). There is again an apparent increase in CH_4 flux during autumn and winter compared to summer, though not at the same magnitude as that seen for CO_2.

5.3.3 Comparisons with benthic metabolism

As the seasonal flux measurements described in Section 5.3.2 were taken during the same sampling periods as the measurements of benthic metabolism described in Chapter 4, the two sets of data can be directly compared. In Figure 5.7, results from all rivers are combined to show average rates of (a) net CO_2 production and (b) net methane production in the light chambers; compared to the fluxes to the atmosphere described above. As discussed in Chapter 4, there were changes in respiration (i.e. O_2 consumption-primary production) between seasons, but these differences were slight compared to changes in CO_2 out-gassing. As shown in Figure 5.7a, flux to the atmosphere is consistently higher than can be explained by benthic metabolism, with the difference most pronounced in winter, when approximately 13 times as much CO_2 is out-gassed than is produced in the sediment. During spring and summer, approximately twice as much is out-gassed than is produced locally in the sediment.

The data summarised in Figure 5.7b show that for CH_4, the converse is true. More CH_4 is produced by net methanogenesis in the sediment than is out-gassed to the atmosphere. The difference is similar in all seasons, with approximately 10 times as much CH_4 emitted from the sediment than is out-gassed.

In Chapter 4 (Table 4.3); Daily (24 hour) net ecosystem metabolism (NEM) was calculated for each river, using the light and dark O_2 benthic consumption data and the number of hours of daylight. Positive values indicated net consumption (i.e. heterotrophy), and reflect respiration rates higher than primary production. In order to estimate daily (24 hour) out-gassing of CO_2 from the seasonal floating chambers data, night fluxes were estimated by adding 30% to daytime flux, informed bythe findings from the intensive spring 2015 deployments that nighttime fluxes were consistently around 30% than daytime, for all rivers. Then, the proportion of CO_2 out-gassing which could be attributed to NEM could be investigated by each individual river (Figure 5.8). The Ebble (chalk) and Nadder (sand) measured negative NEM in spring (i.e. net autotrophy) and were excluded from this calculation. The results show there are large variations in the amount of CO_2 out-gassing that can be attributed to NEM. In the Sem (clay) in summer, twice as much CO_2 is produced than is out-gassed; however, all other results indicate between 1-91% of out-gassed CO_2 originates from NEM in the sediment. In Priors (clay), NEM accounts for 81 and 91% of total metabolism in spring and summer, respectively. This indicates that most of the out-gassed CO_2 is derived from NEM, rather than other inputs. However in autumn and winter, NEM is reduced to 17 and 5% of total flux, respectively. A similar though less extreme pattern is seen in the rest of the rivers, with larger proportions of total out-gassing being accounted for by local metabolism in the summer, and very little in winter. The lowest proportion of out-gassing accounted for by benthic NEM is seen in the Wylye in winter, when it is only 0.9% of the total flux.

These results show that only a proportion of the CO_2 out-gassed by the rivers can be explained by benthic metabolism. However, the 24 hour dataset from the spring 2015

sampling campaign showed that there was a difference between average day and night CO_2 flux, with 30% more CO_2 out-gassed during the night compared with the day. For each river, the difference between day and night flux was calculated, and plotted against the primary production for that river (Figure 5.9). Although these data sets were collected two years apart, the similarity in the daytime out-gassing measured by the spring 2015 campaign and the spring and summer seasonal campaigns suggests they are reasonably comparable. Figure 5.9 shows that the reduction in CO_2 out-gassing during the day is well explained by the magnitude of primary production measured in the benthic sediment of the rivers.

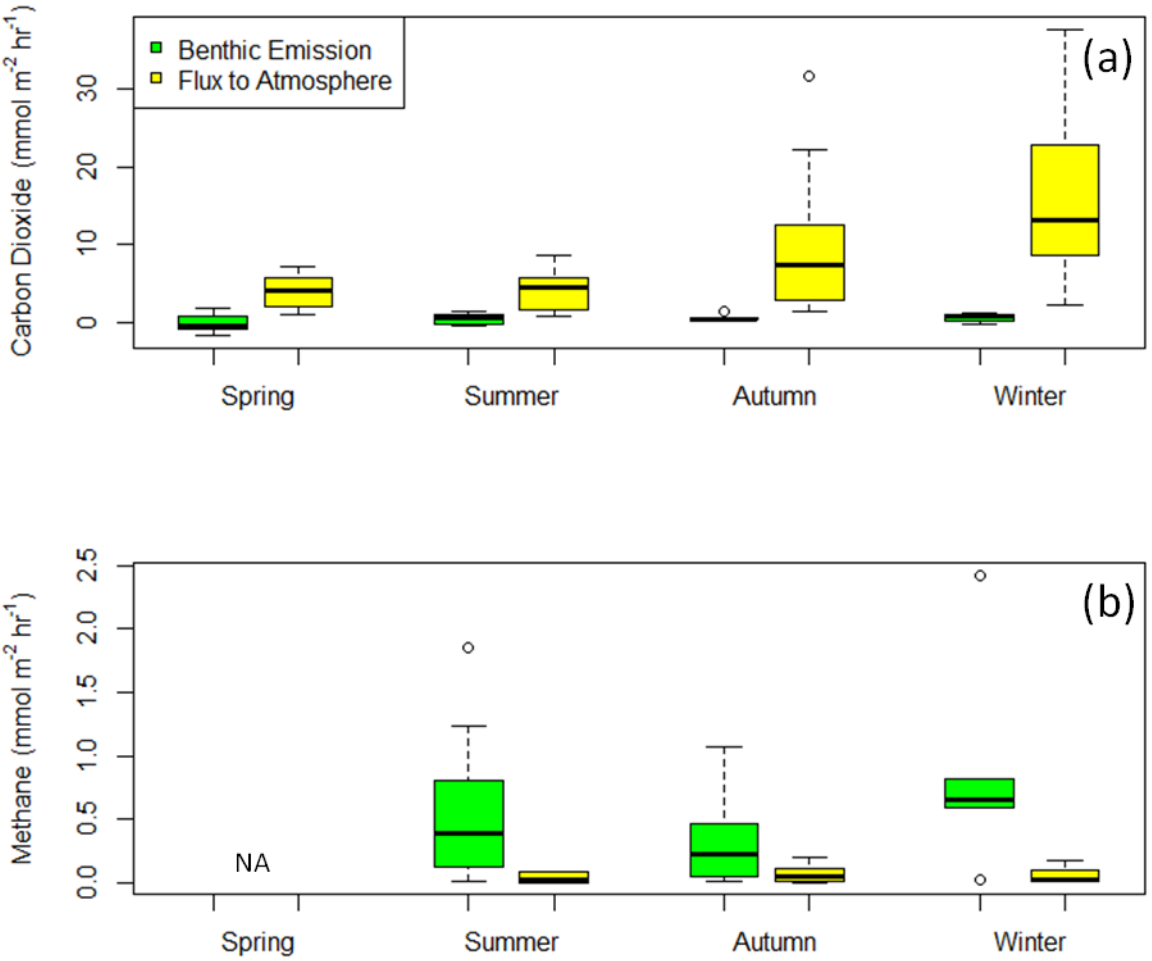

Figure 5.7: Comparisons between emissions of **(a)** carbon dioxide and **(b)** methane from the benthic sediment (green) and the flux from water to atmosphere (yellow). Box plots are combination of all rivers in the study, except those not measured during autumn and winter due to flooding (described elsewhere). Results are from seasonal studies, during which concurrent measurements of benthic metabolism and flux to atmosphere were taken. Measurements of methane were not collected during the spring campaign. Benthic emission measurements are those taken with the light chambers.

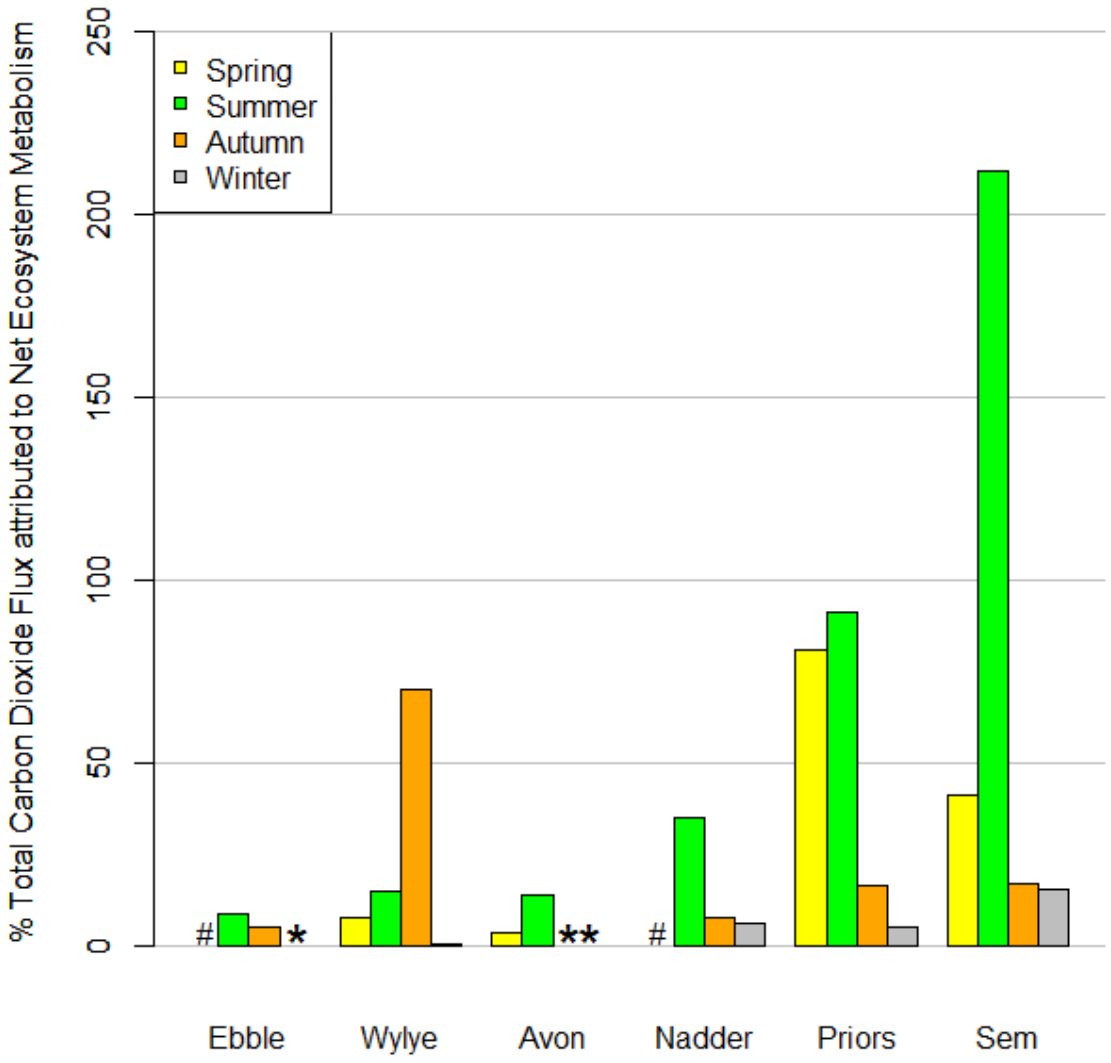

Figure 5.8: Estimated percentage of total CO_2 out-gassing that can be attributed to net ecosystem metabolism. Net ecosystem metabolism rates from Chapter 4 (Table 4.3). Daily flux rates from daytime seasonal floating chamber deployments. In order to approximate total 24 hour out-gassing, night fluxes were estimated by adding 30% to daytime flux due to findings from intensive spring 2015 deployments. * No measurements were taken in the Avon in autumn, or the Ebble or Avon in winter. # The Ebble and Nadder measured negative NEM in spring (i.e. net autotrophy) and were excluded from this calculation.

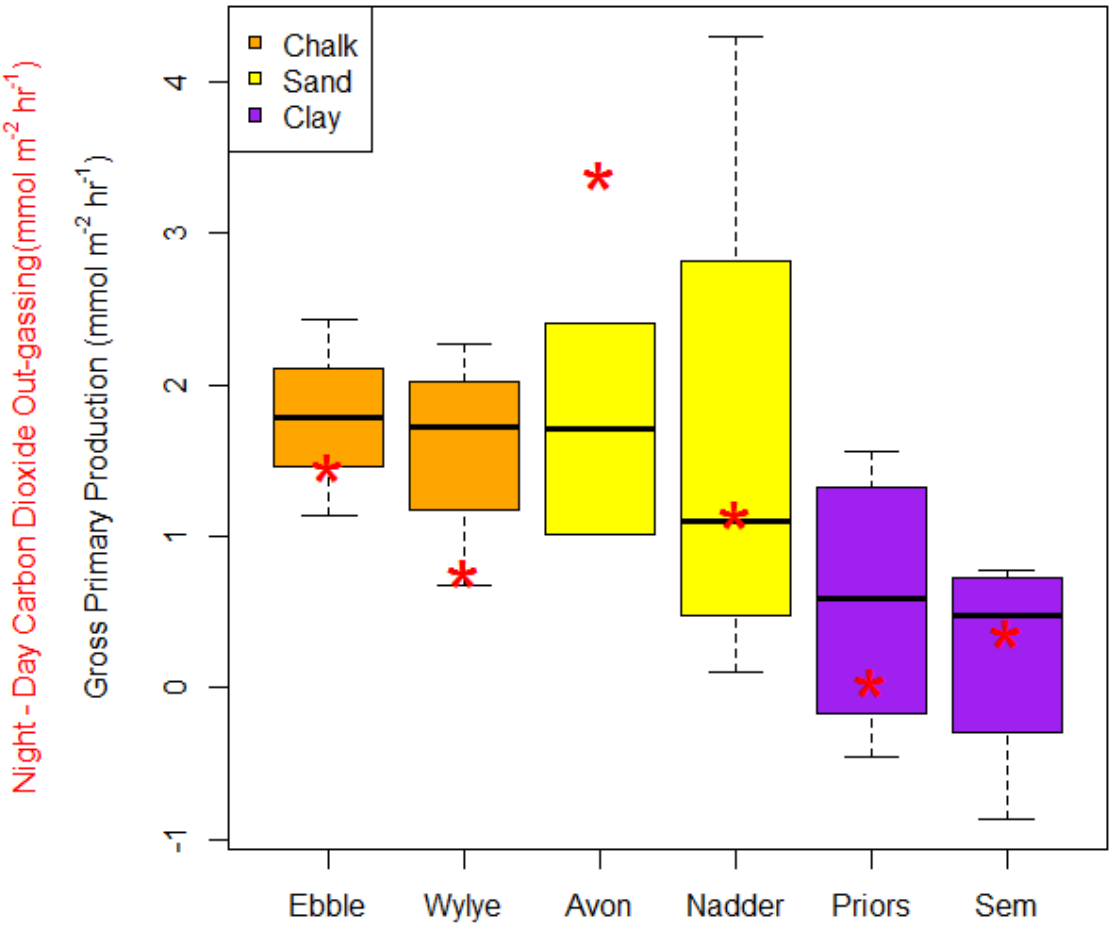

Figure 5.9: Box plots are estimates of primary production in each river from the benthic chamber data in Chapter 4. Red asterisks are the difference between day and night CO_2 out-gassing, measured by floating chamber during spring 2015.

5.3.4 Comparisons with hydrology

For the seasonal study, CO_2 and CH_4 out-gassing were compared with hydrological data that had been collected as described in Section 2.4. There was a large variation in rainfall during the seasonal campaign, as was discussed in Section 2.3. The spring and summer were characterised by very dry conditions with lower than average rainfall. Consequently, water levels and rate of discharge in the rivers were low, and they were under base flow conditions. However during autumn, and particularly winter, heavy and sustained rainfall caused river water levels and rate of discharge to increase markedly at all rivers. This included both base flow and quick flow input of water, though due to the sustained nature of the rainfall base flow contribution was large; i.e. most water was from groundwater input rather than surface runoff during the measurement periods. As shown in Figure 5.10, this range in discharge correlated well with the range in CO_2 and CH_4 out-gassing throughout the year. For CO_2, a linear relationship between discharge and flux was found (r^2 = 0.56, p<0.0001). For CH_4 less data were available, and no relationship was found between flux and discharge.

Regarding both CO_2 and CH_4 out-gassing, the chalk and sand rivers were more variable, and flux measurements responded more strongly to changes in discharge than did the clay rivers (Priors and Sem).

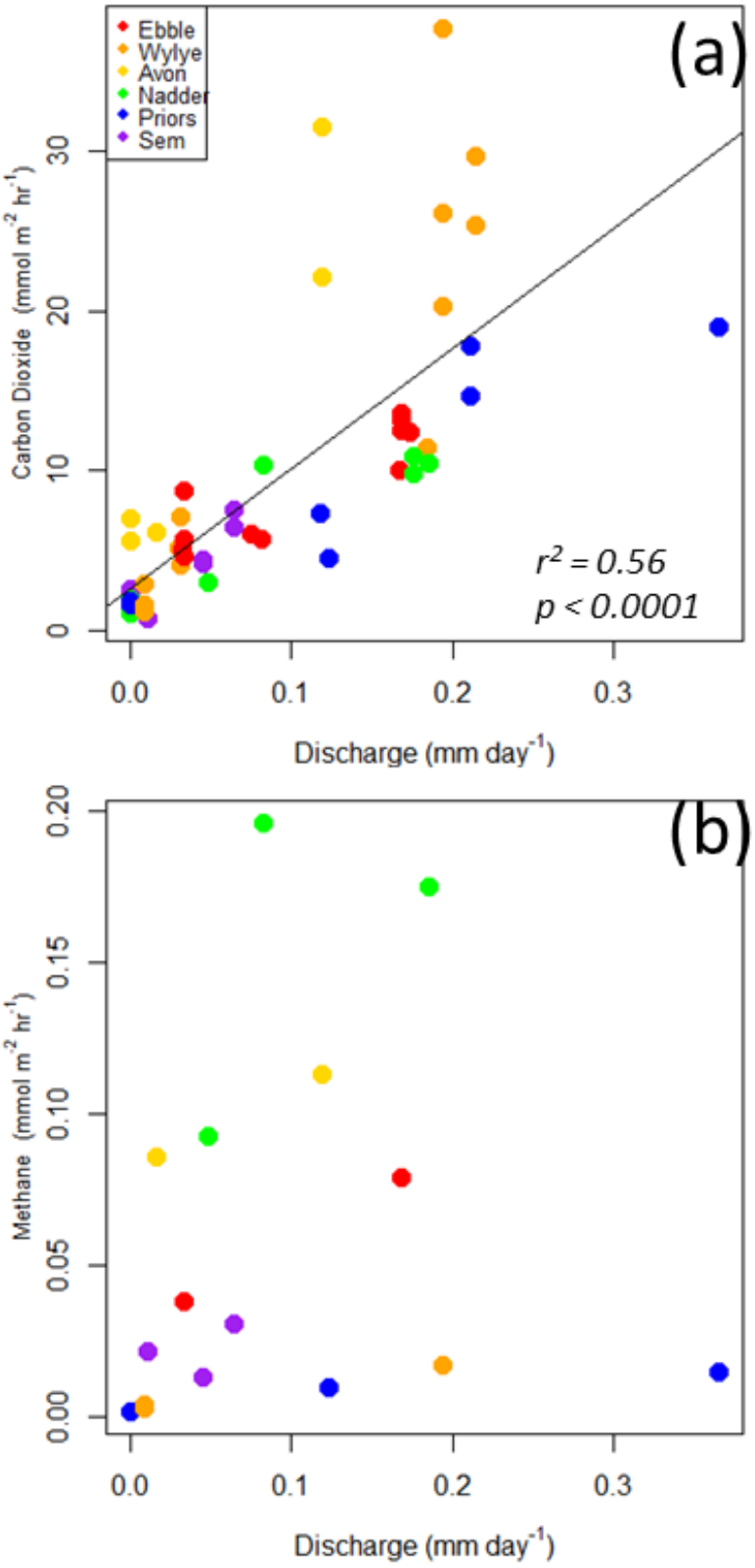

Figure 5.10: Relationship between **(a)** carbon dioxide and **(b)** methane and discharge. Results are from seasonal study, which incorporated periods of very low rainfall (summer 2013) as well as high rainfall and flooding (winter 2014). No samples were taken for methane in the spring; and no samples were taken for either gas for the Ebble and Avon in winter.

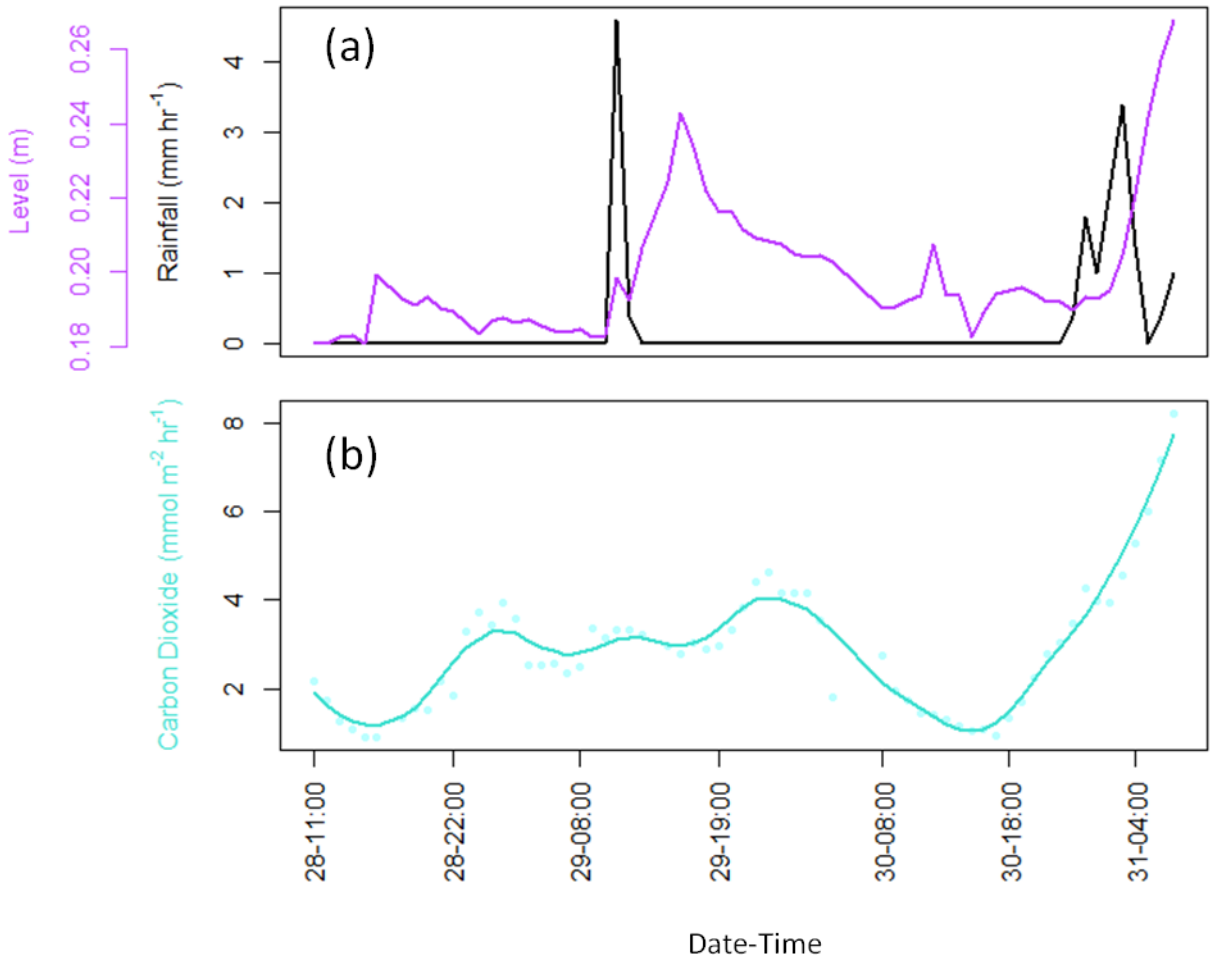

Figure 5.11: (a) Black line shows total hourly rainfall and purple shows hourly average water level (relative to the height of the sensor) during the floating chamber deployment at the Nadder (sand) during the spring 2015 campaign; and **(b)** hourly average carbon dioxide flux during the same time period. Dots represent each flux measurement; line is data with loess (locally weighted regression) smoothing.

Given that the seasonal data suggested a relationship between CO_2 flux and discharge, the data from the intensive spring campaign was also investigated to find if there was a rapid response to rainfall events that could be seen in the data. During that campaign, there were not significant amounts of rainfall during the deployments, with one exception at the Nadder (sand). There was heavy rainfall twice, the second event more prolonged than the first (Figure 5.11a, black line). Both rainfall events were followed shortly by an increase in water level (Figure 5.11a, purple line). There is an apparent response in CO_2 out-gassing (Figure 5.11b),

though both times the response is rapid and may be connected to the rainfall rather than the following increase in discharge. The first rainfall event occurs on the second morning of deployment (29th May), and appears to be associated with a lack of a daytime trough in CO_2 emissions (compared to those on the 28th and 30th, which occur during the early afternoon). The second, larger, rainfall event occurred overnight. CO_2 was already rising due to the diel cycle but got a lot higher than previous maximums. Average flux for the morning (7 to 11 am) of the 29th May was 2.9 (±0.1) mmol $m^{-2}hr^{-1}$, for the 30th May was 2.1 (±0.1) mmol $m^{-2}hr^{-1}$. But for the 31st May, after the rainfall event, it was 8.2 (±0.4) mmol $m^{-2}hr^{-1}$.

5.4.1 Diel and geological influences on CO_2 out-gassing

Diel differences in CO_2 out-gassing were found, with the highest fluxes generally during the night, peaking around midnight. Whilst all rivers were a net source of CO_2, with very few measurements of net CO_2 flux into the water, lowest out-gassing was usually near midday. This was consistent across all rivers, regardless of geology. On average, 30% more CO_2 was out-gassed during the night compared with the day. This difference could be explained by primary production utilizing the CO_2 (Figure 5.9) and so decreasing the concentration in the water column, which would be highest at midday (Odum 1956), corresponding with the lowest flux rate. Additionally, increasing water temperature throughout the day could cause greater out-gassing in the afternoon (Peter *et al.* 2014). This is similar to other measurements of diel variation in CO_2 out-gassing, e.g. Peter *et al*. (2014) found a difference of up to 80% in a similar system. However, it is perhaps surprising that all the rivers showed a very similar reduction in CO_2 out-gassing during daylight: as measured in Chapter 4, the clay rivers have lower rates of primary production than the chalk and sand rivers due to the low light penetration. Additionally, vegetation present in the chalk and sand rivers would increase GPP rates still further in these rivers compared to the clay. These 24 hour measurements were only carried out during the spring; it would be expected that diel variations in CO_2 out-gassing would be reduced in winter due to the lower levels of GPP (such as those presented in Chapter 4); causing a reduction in the amount of CO_2 taken up by autochthonous organisms. Peter *et al.*(2014) found a reduced diel cycle in winter compared to summer in an Alpine stream; however in this case the reduction was due to the difference in nighttime maximum, rather than daytime minimum fluxes.

These diel patterns in out-gassing were not seen in the CH_4 measurements, with no clear difference between average day and night flux and no regular pattern in the diel curves. This is contrary to what was found in Chapter 4 when measurements of net CH_4 flux showed increased production from the sediment in dark chambers, approximately double that in the light. It is important to note that, as with CO_2, the fluxes of CH_4 measured by the floating chamber experiments in this chapter are not only going to be dependent on fluxes from the sediment; but also from CH_4 transported from the surrounding terrestrial catchment, discussed below, and plant-mediated transport (Laanbroek 2010). Methane flux is much less well studied in rivers and streams than is CO_2, and ebullition and plant mediated flux are particularly poorly represented (Bastviken *et al.* 2011). Additionally, methanotrophy and methanogenesis can occur in the water column. Although traditionally thought to be strictly anaerobic, significant methanogenesis has been found in oxygenated water in lakes(Grossart *et al.* 2011; Bogard *et al.* 2014), although this is unlikely in the shallow, flowing water of the study rivers here. On the contrary, in this study CH_4 out-gassing rates were far lower than those from sediment to water measured in Chapter 4. This may be due to significant methane oxidation by methanotrophic bacteria in the water column; one study in a lake estimated that 3-5 times as much CH_4 is oxidised in the water column than is out-gassed, and that 80% of CH_4 released from the sediment is oxidised in the water column (Kankaala *et al.* 2006).

These processes may therefore have masked any differences between day and night production rates. One study using eddy correlation found higher CH_4 fluxes during the night than the day (Podgrajsek*et al.* 2014);however, this was in a lake rather than a river or stream, where the average night-time flux was 1.08 mmol $m^{-2}hr^{-1}$, far higher than the average found here of 0.02 mmol $m^{-2}hr^{-1}$. Therefore in that study, the flux to the atmosphere may have been more indicative of the flux from the sediment, with less dilution effect therefore the diel cycle was seen. Atmospheric stratification, which is stable at night and unstable during the day is

also suggested as a driver in this diel CH_4 change, in that study and other, terrestrial studies (Podgrajsek*et al*. 2014; Baldocchi *et al*. 2011).

Variations in the magnitude of fluxes between rivers were seen, but there was no clear gradient with BFI, for CO_2 or CH_4. There are general trends with geology; both CO_2 and CH_4 emissions were typically highest in the sand rivers (Nadder and Avon) and the Ebble (chalk) (Table 5.1 and Figure 5.6). However, the other chalk river (Wylye) had some of the lowest fluxes measured, along with the clay rivers (Priors and Sem). During both the intensive campaign and during spring and summer of the seasonal campaign, the highest CO_2 fluxes are seen in the Ebble and the Avon. This does not correlate well with the benthic chamber measurements of ER and GPP, which show highest respiration (and so most CO_2 production) in chalk and clay rivers.

The high CH_4 fluxes from the water to the atmosphere in the sand rivers may be due at least in part to lower rates of methanotrophy; at least compared to the clay rivers. Methanotrophy typically occurs in a thin layer of oxygenated sediment on the riverbed surface.Due to faster rates of gas transport through the larger, less impacted sand particles compared to clay, this could cause higher CH_4 fluxes in sand overall. In addition, the atmospheric fluxes from the sand rivers would include CH_4 derived from plant-mediated transport, from the macrophytes present (Figure 4.3).

If fluxes were dependent, entirely or predominantly, on benthic production of these gases it would be expected that they would be related. With the exception of the highest CH_4 fluxes being seen in the sand rivers, this was not the case. Instead, it is clear there are other factors controlling the flux of these gases from river to atmosphere, such as flow rate, water column metabolism or other sources of CO_2 and CH_4. These factors will be discussed in more detail below.

5.4.2 Benthic metabolism modulates out-gassing, but cannot explain the full magnitude.

Comparison with the measurements of benthic carbon metabolism described in Chapter 4 show fluxes of CO_2 were modulated by microbial respiration and production in the sediment; with the diel fluctuations in out-gassing largely explained by net differences in net dark and light CO_2 benthic production. However benthic metabolism could not account for all of the CO_2 out-gassed by the river to the atmosphere. Particularly during autumn and winter and particularly in the chalk rivers, CO_2 out-gassed far exceeded that produced by the net heterotrophy of the sediments described in Chapter 4.

Instead, the primary source of out-gassed CO_2 appears to be from lateral transport of water and dissolved gases of terrestrial origin, from the surrounding catchment. Studies which consider both out-gassing and local metabolism concurrently are sparse and none are known which also consider CH_4; although one recent meta-analysis estimated rates of benthic production and out-gassing of CO_2 in rivers and streams in the USA (Hotchkiss *et al.* 2015). That study estimated that 28% of the CO_2 out-gassed could be accounted for by local metabolism; however discussion of spatial and temporal variability is limited. The average found in this study is virtually identical: 30%; however large variation was found around this, both between different rivers and between seasons (Figure 5.8). In the Wylye in winter, less than 1% of out-gassing could be explained by benthic metabolism (NEM), whilst in the two clay rivers (Sem and Priors) in spring and summer, NEM could explain the majority of out-gassing. This variation appeared to be due in part to spatial variation (the underlying geology of the rivers) and temporal variation (the extremes of weather experienced between seasons during the experiments).

Here it was found that in rivers with lower BFI (clay), a higher proportion of CO_2 out-gassing could be attributed to local metabolism. However with the exception of the Wylye in autumn, where local metabolism contributed 70% of out-gassed CO_2, benthic metabolism in

chalk and sand rivers did not contribute more than 35% of the total CO_2 out-gassed. The Wylye was similarly anomalous in measurements of benthic metabolism in Chapter 4, where the highest rate of respiration was measured in the autumn, contrary to the other rivers which all had the highest rates in spring or summer. Therefore a higher proportion of locally derived CO_2 in the total flux may be due to this higher local respiration measured in this case. Additionally, discharge measured at the Wylye in autumn was similar to that in spring and summer, contrary to most other rivers where it was much higher due to the high rainfall experienced. These findings suggest that groundwater input, highest in chalk and lowest in clay, may be a significant source of CO_2 to these rivers. In spring, the Ebble (chalk) and Nadder (sand) recorded net autotrophy over a 24 hour period; implying that at these times, some of the CO_2 received by the system is fixed by local primary producers.

It is known that CO_2 can be highly enriched in groundwater (Macpherson 2009), particularly in chalk landscapes due to microbiologically induced calcite precipitation as well as other biological activity (Griffiths *et al.* 2007). Recent research has shown that groundwater discharge during floods can be a major driving factor in CO_2 out-gassing from freshwaters (Atkins *et al.* 2013). Hotchkiss *et al*. (2015) propose that the source of excess out-gassed CO_2 (not explained by benthic metabolism) is principally derived from groundwater, and that the input is predominantly inorganic carbon compounds rather than organic carbon which is then metabolised to CO_2 in the water column. This is because CO_2 can be out-gassed more rapidly than organic carbon, which is dependent on biological action first. The results presented here support that view, as CO_2 emissions markedly increase in line with increasing discharge in autumn and winter, when it may be expected that biological activity needed to break down organic carbon would be limited due to low temperatures. Chapter 4 shows lowest reach-scale ER in winter, and positive correlation of ER with water temperature.

As well as the increased input of dissolved gases from groundwater, it may be suggested that physical changes due to increased discharge would increase the flux of dissolved gases

from the river to the atmosphere. Long *et al.*(2015) also conclude that ecosystem metabolism is not the primary control on CO_2 flux, and suggest that it is changes in convection and turbulence of the water that renews the surface boundary layer, allowing more CO_2 to come into contact with the water surface and so be out-gassed. This may be the cause of the short-term increases in CO_2 out-gassing measured in the Nadder during the intensive campaign (Figure 5.11). The rapid response of CO_2 out-gassing to the rainfall may have been caused by turbulence due to the rain disrupting the boundary layer or possibly increased wind speed. This short-term effect in out-gassing was not an initial objective of the research and could be investigated further with more measurements, including better resolving how long after rainfall the change in CO_2 out-gassing occurs and how long the effect is sustained after rainfall.

5.4.3 Significant rainfall and flooding events have a substantial effect of CO_2 out-gassing from rivers.

Seasonal changes in CO_2 out-gassing were large, and coincided with the exceptional weather conditions that occurred whilst the seasonal deployments were carried out. As these weather conditions were not common, particularly for the autumn and winter, it cannot be said that this is a regular seasonal pattern. Instead, repeated rainfall events, resulting in sustained high discharge, caused a notable increase in CO_2 emissions during this study period (Fig 5.10). This was not found to be the case with CH_4 emissions, though a reduced number of measurements were taken of CH_4 so less confidence can be stated regarding this, given the inherent heterogeneity of CH_4 emissions and the apparent difference between rivers and geologies. Having said that, some seasonal changes were seen in CH_4 out-gassing, but these were not as large as the change in CO_2 and did not correlate well with discharge. Instead, the rivers with the highest fluxes (Ebble, Nadder and Avon) were consistently high; whilst the other rivers (Wylye, Priors and Sem) were low across all measurement periods, not going above 0.05 mmol $m^{-2}hr^{-1}$. However all rivers were reliably net sources of CH_4 to the

atmosphere: as with CO_2, fluxes below zero were seldom measured. The greater rate of flux from the sediment compared to the flux to the atmosphere, described above, and the potential for significant water column methane oxidation (Kankaala *et al.* 2006) suggest that for CH_4 out-gassing, biological controls may be more important, in contrast to CO_2 out-gassing where physical factors of increased groundwater input and turbulence are the main controls.

The key finding here, of rates of CO_2 flux more than doubling during periods of high flow, may have implications for future climate feedbacks. The winter of 2013-14, during which the autumn and winter experiments were undertaken, was characterised by extreme rainfall and storm events. As shown in Figure 2.3 (Chapter 2), this included the highest rainfall recorded in Southern England in 100 years during the month of January 2014. This was due predominantly to the atmospheric circulation which occurred, and could not be conclusively linked to anthropogenic forcing at the regional scale (Christidis & Stott 2015). However, a warmer atmosphere can hold more water (Allan & Soden 2008), and wetter winters are predicted to become more common during the second half of the 21st century (IPCC 2013). If rates of CO_2 out-gassing are consistently higher with increased rainfall events, this may lead to increased CO_2 out-gassing in the future. However, it is more likely that the increased out-gassing represents 'flushing' of CO_2 that has accumulated in the surrounding soil into the water column. This may mean that overall, no increase in net CO_2 emissions occurs.

The results in this Chapter show that CO_2 out-gassing is consistently greater than can be explained by benthic metabolism, particularly in chalk rivers. This effect is exacerbated by higher discharge rates, in all the rivers. This suggests the high CO_2 in groundwater (particularly in chalk landscapes), derived from terrestrial metabolism and weathering, is a source of out-gassed CO_2 and greater volumes are mobilized with the higher discharge during periods of high discharge. As such, it reiterates the importance of including freshwaters in models of the global carbon cycle, and the importance of tracing the path of carbon fixed by terrestrial primary production. If measurements of total production are greater than those of respired

CO_2 emitted to the atmosphere, it is not correct to assume the surplus carbon is stored in the terrestrial sphere.Instead transport to, then emission from, freshwaters may be a significant pathway for terrestrially-derived carbon. Having said this, there is also some evidence of a physical increase in CO_2 out-gassing due to turbulence (Figure 5.11). These short-term increases in flux would not necessarily be an additional source of CO_2, but an out-gassing that takes place further upstream than it otherwise would. However, short-term increases in turbulence would not cause the sustained increases of CO_2 flux seen in the seasonal study (Figure 5.6), which suggest the input of an additional carbon source from the surrounding larger catchment. These different controls on emissions, and differences between geologies (Figure 5.8), suggest that simply estimating fluxes from the concentrations of CO_2 in the watertogether with estimates of gas transfer velocities may not be accurate. Further investigation of the long and short-term effects of rainfall events and discharge on CO_2 out-gassing, and how these compare with benthic metabolism, are needed.

5.5 Conclusion

This chapter has examined both CO_2 and CH_4 out-gassing from rivers, over diel and seasonal timescales. Using the six rivers of the study allowed variations in flux due to geology to be investigated, as well as allowing comparisons between atmospheric flux and benthic metabolism to be made.

The discovery that benthic production of CO_2 and CH_4 is of only minor consequence for the eventual out-gassing at the river surface is a notable finding, and reinforces the importance of the connection between terrestrial and freshwater ecosystems for the carbon cycle.

This study is unique in directly measuring both fluxes from the sediment and the river surface, an in incorporating a range of geologies and seasons. This has allowed the magnitude of variation between different rivers and times to be considered, and shown that these can be large, particularly in the case of extreme weather events.

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6. Conclusions and Further Work

6.1 Overview

This thesis has investigated carbon dioxide (CO_2) and methane (CH_4) metabolism in rivers across a range of scales: from laboratory based, potential measurements, to *in situ*, reach scale estimates and lastly measuring the actual contribution of CO_2 and CH_4 from the rivers to the atmosphere. Measuring these processes in the same study rivers but from different viewpoints has enabled the dynamics of CO_2 and CH_4 emissions to be evaluated and comparisons made. Key findings in the potential production results informed on directions to take during the *in situ* studies, in particular with regard to different patch types. Comparison of *in situ* production and atmospheric flux of CO_2 and CH_4 revealed the contribution of benthic metabolism to out-gassing, showing that local production is not the main controlling factor on CO_2 or CH_4 efflux.

6.2 Comparisons of potential and *in situ* measurements of carbon metabolism

The potential measurements of CO_2 and CH_4 production described in Chapter 3 were done under anoxic, laboratory conditions whilst the experiments carried out in Chapter 4 were carried out under conditions much closer to undisturbed, natural conditions in the rivers. One main difference between the two methods was in CH_4 production in sand: the potential measurements showed very low production whilst the *in situ* study recorded rates comparable to those in the clay rivers. Conversely, the chalk rivers under laboratory conditions had high rates of CH_4 production, whilst the *in situ* measurements in chalk were consistently the lowest. These differences may be in part due to the differences in oxygen concentrations: the potential samples were all prepared the same, whilst a difference between oxygen concentrations in the rivers may be expected. Hyporheic exchange flows in permeable sediments, particularly in the chalk rivers, can deliver oxygen to several centimeters into the sediment. Additionally, presence of vegetation, again mostly in the chalk rivers but also the sand rivers, can increase oxygenation of the sediment by translocation by the plant roots. In particular, oxidation of CH_4 by methanotrophs has been shown to be able to match that produced by methanogenesis under optimum conditions (Shelley *et al.* 2015). Methanotrophs would be inhibited by the anoxic conditions in the laboratory study but may thrive when under normal conditions in the top layer of the chalk river sediments.

Respiration, was measured by CO_2 production in the potential measurements and O_2 consumption in the dark chambers in the *in situ* study, No overall effect of river or geology was found for the dark *in situ* measurements, but in the potential measurements the clay rivers had highest production, closely followed by chalk, with sand rivers producing considerably less CO_2. As with CH_4, it is likely that this is due to differences in *in situ* conditions that were not replicated by the potential measurements. It could be that the clay and chalk sediments have a greater store of organic matter that is readily available for respiration, whilst the sand rivers, which usually had high flow rates, are more dependent on the river flow to deliver organic

matter, or other nutrients. In addition, as with the differences in CH_4 production between Chapters 3 and 4, the oxygen concentration may have been important. As the potential samples were incubated under anoxic conditions, respiration in all the rivers would have been lower than would be expected under *in situ* conditions. Again, this may have affected the sand rivers more than the clay and chalk due to their high flow rates and low organic matter content. As such, redox state may vary between rivers and geologies, and be different in the lab and *in situ*. The potential measurements were carried out over a number of days, during which time the samples were kept in the dark. This is different to the *in situ* conditions where the sediments are exposed to light during the day and therefore photosynthesis was able to occur. This could have affected the supply of labile organic matter in the sand rivers, certainly compared to the clay rivers which would be more dependent on lateral transport for organic matter supply.

Considering patch differences in the rivers, the potential and *in situ* measurements do show some similarity, with more CH_4 production in marginal and vegetation sediments than in un-vegetated, main gravel or sand riverbeds, and in particular large differences are seen once again in the Wylye. Increased CO_2 production is recorded in the potential samples is seen in the *in situ* results; although not always as high as the differences seen in the potential data, and not in every chalk and sand river. In the Ebble, respiration rates in the fine, marginal sediment are far more variable than those in the main un-vegetated gravel, but are not clearly larger. In Chapter 3, it was concluded that small-scale differences in sediment characteristics could be better predictors of CO_2 production than geology.This indicates that there may be a significant amount of heterogeneity in characteristics of the fine sediments in this river.

Seasonal differences in production were similar in both methods, with lower respiration measured in winter but no seasonal differences in CH_4 production in either method. Temperature was found to be important in CO_2 production in both methods, but for CH_4 production temperature was only found to be of significance in the laboratory measurements.

Again, this may suggest the influence of methanotrophy under conditions found *in situ*, and their ability to respond to higher production by methanogens with increased rates of oxidation.

6.3 Rivers and their relationship to the surrounding terrestrial landscape

A central theme of this thesis was the inclusion of a range of underlying geological characteristics of the rivers. Underlying geology impacts the sediment characteristics, vegetation and hydrological connections to the surrounding terrestrial landscape. It was hypothesized that these differences could in turn cause differences in metabolism in the benthic sediment, and so differences in CO_2 and CH_4 fluxes from the riverbed to the water and from the water to the atmosphere. Using base flow index (the proportion of water that comes from groundwater, BFI) to quantify geology was considered for Chapter 4 as there were only 2 replicates from each geological group.

Some differences were found between geology, however there was not a linear relationship with BFI; instead broad similarities were found, for example potential production of both CO_2 and $CH_4$was high in both chalk and clay rivers. The only measured rate with a linear relationship with BFI was GPP.

Whilst geology was a useful predictor of some aspects of CO_2 and CH_4 metabolism, a more interesting factor was the local differences, including patch differences and the small-scale heterogeneity found with river reaches, proving the importance of including the full range of small-scale environmental differences and including accurate scaling-up considerations in experimental design.

The finding that CO_2 and CH_4 fluxes from the sediment are not strongly tied to out-gassing prove that connection with surrounding terrestrial processes can be very important. Out-gassing of CO_2 was usually greater than could be explained by local metabolism, indicating delivery of terrestrial CO_2. There were differences between geologies, with chalk rivers consistently out-gassing more CO_2 than was locally produced even under base flow conditions, whilst out-gassing from clay rivers only surpassed local production during periods of exceptional rainfall and high river level.

6.4 Challenges in measuring methane metabolism

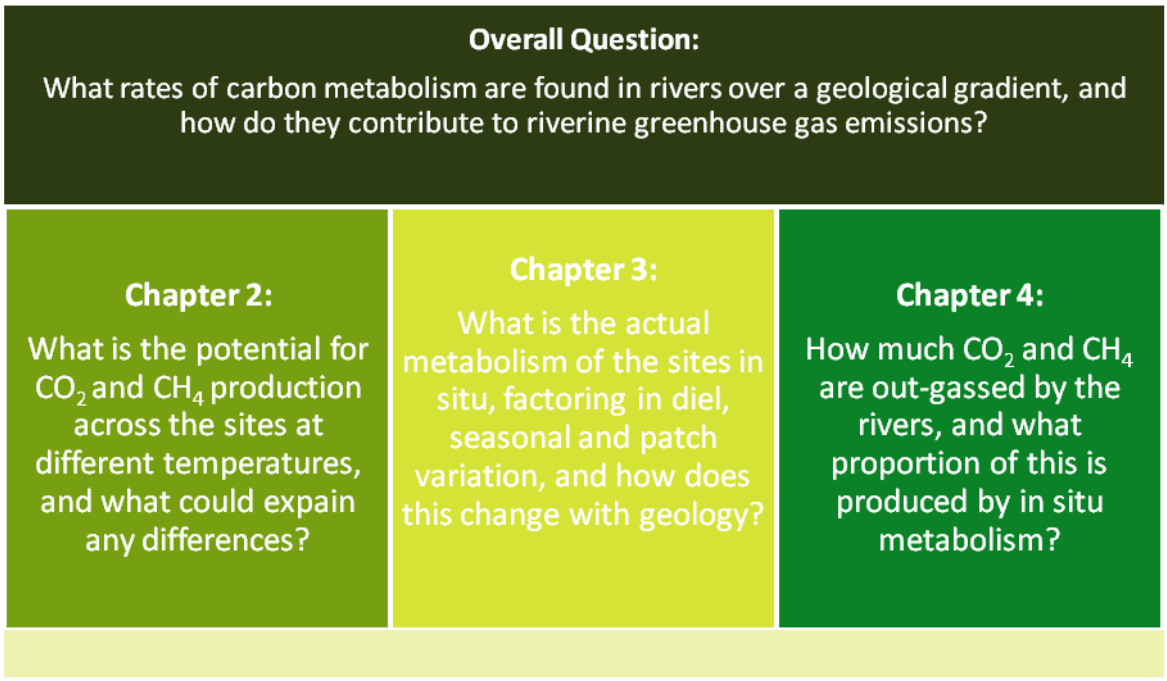
Although only a minor part of the atmosphere, CH_4 has been under increasing attention due to its role in global warming, with 28 times the global warming capacity of CO_2(Myhre *et al.* 2013), and is responsible for 17% of the radiative forcing due to increased concentrations of well-mixed greenhouse gases (Allen 2016). Concentrations in the atmosphere have increased 150% since preindustrial levels, at 1803 ppb in 2011 (IPCC 2013). By comparison, CO_2 concentration has risen by 40% over the same period.

Consequently, inclusion of CH_4 in studies of ecosystem metabolism is necessary, though it remains much less well investigated than CO_2(Stanley *et al.* 2016). However, this is complicated by the difficulties inherent in CH_4, most especially the high variance and heterogeneity of fluxes at small scales. In this study, differences in CH_4 data of as much as 3 orders of magnitude were a recurrent theme, in Chapters 3 and 4 in particular. These variances were seen even within the same riverbed and the same experimental investigations, and subsequently meant that patterns between geology, season or other factors could be difficult to identify. This characteristic of CH_4 is seen in many studies (Dinsmore *et al.* 2013; Stanley *et al.* 2016). The findings in Chapter 3 show that sediment characteristics, such as organic carbon content and particle size, are in some ways more reliable predictors of CH_4 production in riverbeds than larger scale variations such as underlying geology and season. As noted by Stanley *et al.*(2016), within-stream variability can be of similar magnitude to the total variability of estimates of CH_4 fluxes in systems worldwide.

This shows that special care must be taking when designing experiments measuring flux of CH_4, from freshwaters and other environments, to include enough replication and consideration of influencing variables in order to constrain accurate estimates of CH_4 metabolism, and make predictions based on these. The results in Chapters 3 and 4 describe the large differences between patch types in chalk rivers: this is one example of an area where CH_4 estimates are greatly improved by including this variable. The differences measured in

CH_4production between the light and dark in Chapter 4 are an example of an area that needs further investigation.

6.5 Addressing the research questions identified in Chapter 2



6.5.1 Chapter 3: **What is the potential for CO_2 and CH_4 production across the sites at different temperatures, and what could explain any differences?**

Significant production of CO_2 was measured in all rivers, at one average 0.13 µg C g $dw^{-1}hr^{-1}$,and CH_4 production was measured in most though varied between 0 and 541 ng C g $dw^{-1}hr^{-1}$. Some geological differences were identified, with sand river sediments producing less CO_2 and CH_4 than the chalk and clay rivers. However, variations in sediment characteristics could be large within rivers, and were more important than the overall underlying geology in predicting CO_2 and CH_4 production. Both CO_2 and CH_4 production responded strongly to temperature, but the higher temperature dependence of CH_4 has important implications for the ratio of the two gases emitted under warming conditions.

6.5.2 Chapter 4: **What is the actual metabolism of the sites *in situ,* factoring in diel, seasonal and patch variation and how does this change with geology?**

Rates of NEM, ER, GPP and net CH_4 production were calculated for each river reach, and showed expected differences between light and dark O_2 consumption as well as surprising differences in net CH_4 production. Geological differences in GPP were found, with highest rates in the chalk and sand and in spring, but ER was very similar across rivers and geology. Higher net CH_4 production was measured in the sand and clay compared to the gravel bed of the chalk rivers; however patches of fine marginal or vegetated sediment in the chalk rivers were shown to be significant producers of CH_4.

6.5.3 Chapter 5: **How much CO_2 and CH_4 are out-gassed by the rivers, and what proportion of this is produced by *in situ* metabolism?**

All rivers measured net out-gassing of both CO_2 and CH_4, i.e. were net sources of these gases to the atmosphere. However large differences between seasons were found, due to the difference in rainfall and river level. Out-gassing of CO_2 was far higher than could be explained by benthic metabolism, suggesting significant influx of terrestrial CO_2 from groundwater sources. Conversely, CH_4 out-gassing was much lower than what had been measured in the sediment, indicating dilution or substantial methanotrophy in the water column.

6.6 Overall estimates of carbon cycling

In Figure 6.1a, all the sources and sinks of carbon considered in this thesis are included, to estimate the net effect of various key processes, and how they compare. Hexagons represent CO_2 or CH_4 production (blue) and consumption (red). Larger hexagons represent larger production and consumption rates. Measured rates are given in black, whilst estimated rates that were not directly measured are given in red. De-gassing of CO_2 in the study rivers was 1-10 mmol m^{-2} hr^{-1}. When this is compared to rates of respiration (2 ±0.1) and photosynthesis (1 ±0.2), it shows the 'missing' carbon source discussed in Chapter 5. It is suggested here that much of this carbon is derived from influx from lateral transport (0-8 mmol m^{-2} hr^{-1}).

Conversely, de-gassed CH_4 was 0.002-0.05 mmol m^{-2} hr^{-1}, compared to a much higher rate of net methane production in the sediment of 0-2 mmol m^{-2} hr^{-1}. In figure 6.1a, this difference is suggested to be explained at least partly due to methane oxidation in the water column (up to 0.05 mmol m^{-2} hr^{-1}).

Figure 6.1a includes illustration of plants in the riverbed. Although rates of plant respiration, photosynthesis and plant-mediated transport were not measured in this study, they should be expected to be significant and thus would need to be included in any whole-river estimate of carbon metabolism.

The main controlling factors driving rates are considered in Figure 6.1b. The text boxes in this figure also summarises the key differences found between the different geologies. In short, the best predictor of production in the sediment was organic carbon content, which was typically high in the clay (lowest BFI) and the chalk (highest BFI), but low in the sand (intermediate BFI). As such, a simple linear relationship between BFI and CO_2 and CH_4 production did not exist. Rates of photosynthesis in the sediment however were highest in the chalk and lowest in the clay due to the difference in turbidity in the water column. The magnitude of de-gassing of CO_2 was not predicted by geology, although the proportion of the total de-gassed was influenced by geology as described in Section 5.4.2.

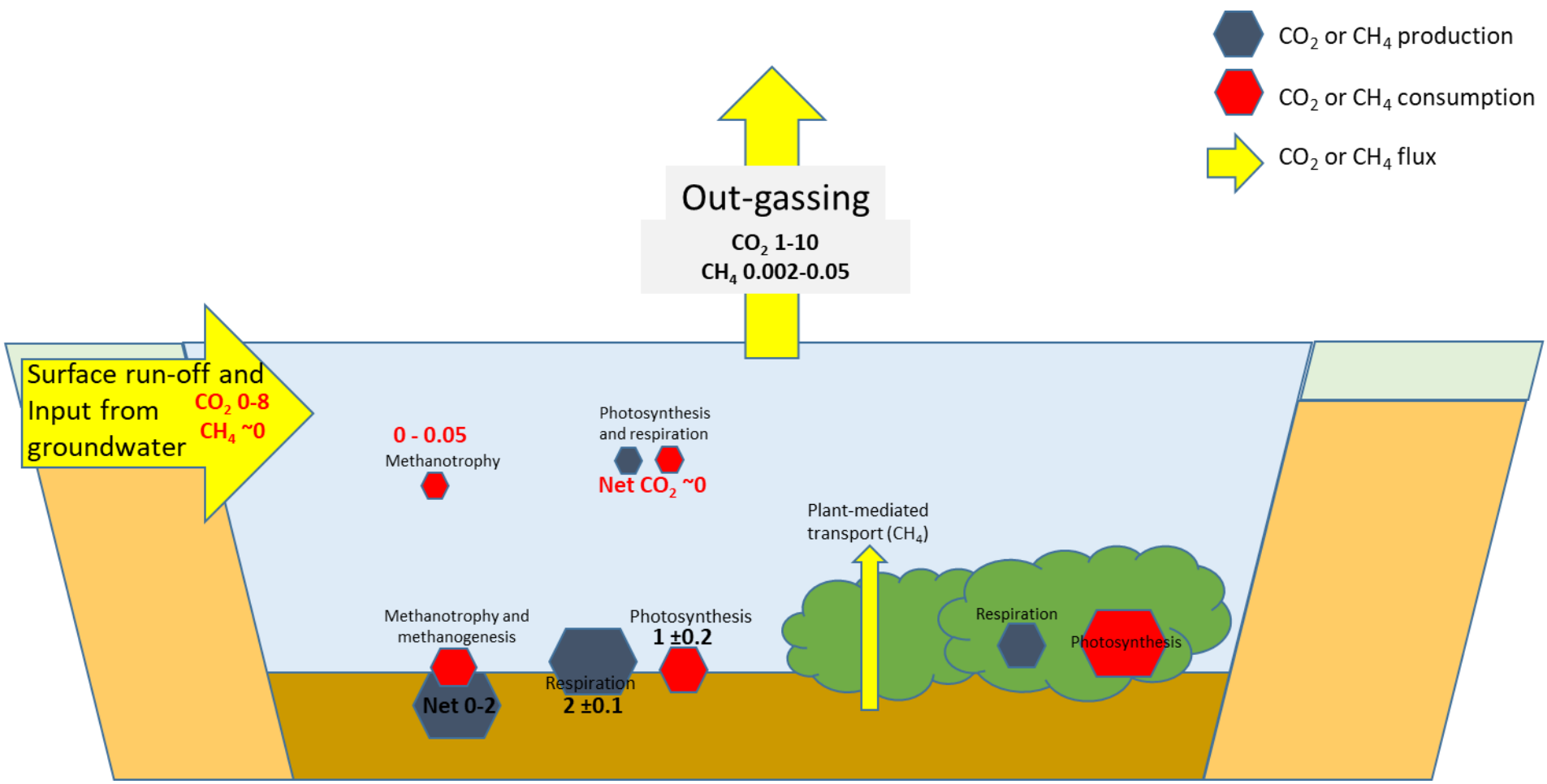


Figure 6.1a: Diagram of all sources and sinks of CO_2 and CH_4 considered in this work. Numbers represent production in mmol m^{-2} hr^{-1}. Figures in black indicate numbers derived from the experiments described in the previous chapters. Figures in red are estimated sinks and sources.

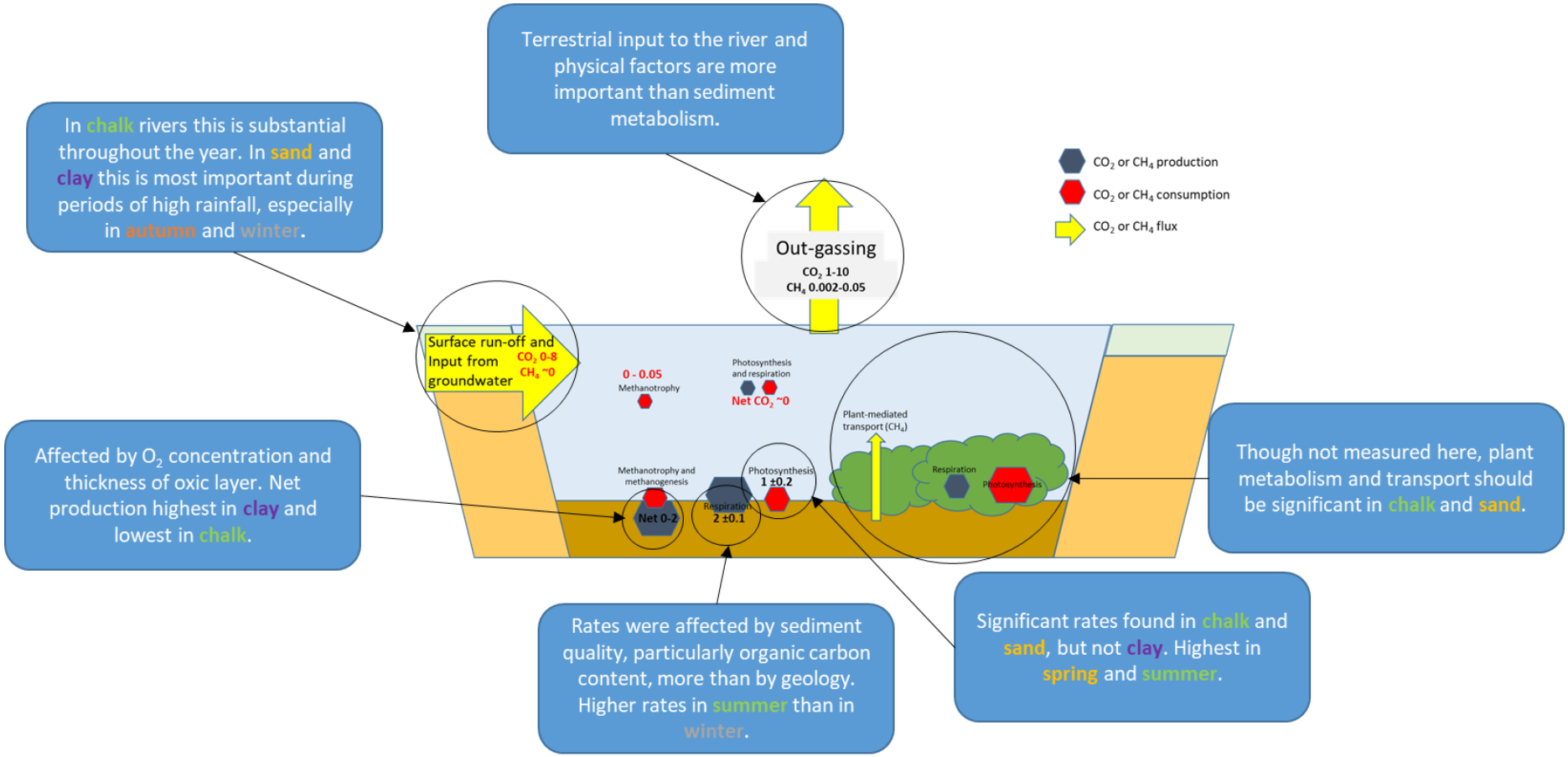


Figure 6.1b: As Figure 6.1a, above, but with comments of key findings of factors which control rates.

6.7 Implications of this study

Key findings:

- Differences in CO_2 and CH_4 production between geologies are seen, but within reach variation can be of greater importance.
- The strongest predictor of both CO_2 and CH_4 production when considering intra- and inter-reach variability is organic carbon and nitrogen content of the sediment.
- Temperature dependency of CO_2 and CH_4 production in river sediment is conserved across geologies and seasons.
- The higher temperature dependency of CH_4 compared with CO_2 noted in other studies (Yvon-Durocher *et al.* 2010) was found in the potential measurements, but no overall temperature effect could be measured when considering net CH_4 production *in situ*.
- Net CH_4 production *in situ* is approximately twice as high in the dark compared to the light, although there is large variation in this.
- Terrestrial and hydrological influences are more important factors in river CO_2 and CH_4 out-gassing than benthic metabolism.

Further investigation of CH_4 production in rivers, and the controlling factors on this, is needed, given the importance globally of CH_4 emissions and the large variation at small scales. The increased production of CO_2 and especially CH_4 in fine sediments has implications for land use management, which may be more important than temperature increases in future and so should be considered in land use planning and monitoring. The increased CO_2 out-gassing during periods of high rainfall demonstrates the necessity of considering rivers and other freshwaters in carbon cycle models and monitoring.

6.8 References

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7. Appendix

7.1 Potential Production of Carbon Dioxide and Methane

Tables 7.1 and 7.2 show the average rate of production of CO2 and CH4 in the potential measurements described in Chapter 3. These results are summarized in Figure 3.3, and discussed in that Chapter.

		Carbon Dioxide µgC g dw^{-1} hr^{-1}											
		5 ˚C				10 ˚C				22 ˚C			
	River	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*
Chalk	Ebble	0.06	*0.02*	0.04	*0.01*	0.07	*0.01*	0.04	*0.01*	0.17	*0.03*	0.09	*0.01*
	Wylye	0.10	*0.02*	0.06	*0.01*	0.10	*0.01*	0.09	*0.03*	0.25	*0.05*	0.12	*0.02*
	Wylye Patch A	0.32	*0.05*	0.37	*0.08*	0.60	*0.18*	0.51	*0.14*	1.08	*0.22*	1.27	*0.28*
	Wylye Patch B	0.07	*0.01*	0.12	*0.02*	0.09	*0.01*	0.14	*0.03*	0.44	*0.16*	0.33	*0.04*
	Rushall-chalk	0.11	*0.03*	0.07	*0.02*	0.18	*0.06*	0.07	*0.01*	0.54	*0.18*	0.14	*0.03*
Sand	Avon	0.06	*0.01*	0.01	*0.00*	0.10	*0.01*	0.01	*0.00*	0.19	*0.02*	0.05	*0.01*
	Avon Patch A	0.06	*0.01*	NA	*NA*	0.08	*0.01*	NA	*NA*	0.19	*0.04*	NA	*NA*
	Avon Patch B	0.06	*0.02*	0.05	*0.01*	0.08	*0.02*	0.08	*0.01*	0.17	*0.04*	0.17	*0.01*
	Nadder	0.05	*0.01*	0.05	*0.02*	0.06	*0.01*	0.05	*0.01*	0.14	*0.01*	0.11	*0.03*
	Rushall-sand	0.10	*0.04*	NA	*NA*	0.10	*0.03*	NA	*NA*	0.18	*0.02*	NA	*NA*
Clay	Priors	0.15	*0.02*	0.06	*0.02*	0.23	*0.03*	0.07	*0.02*	0.76	*0.11*	0.18	*0.04*
	Sem	0.08	*0.01*	0.06	*0.02*	0.12	*0.01*	0.09	*0.03*	0.26	*0.03*	0.18	*0.08*
	Cools	0.07	*0.02*	0.11	*0.01*	0.10	*0.02*	0.16	*0.02*	0.27	*0.03*	0.31	*0.03*

Table 7.1: Rate of potential production of carbon dioxide in the nine study rivers in summer and winter. Results are averages (*n = 5*), of each of the three temperatures incubations were conducted at.

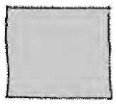
		Methane ngC g dw^{-1} hr^{-1}											
		5 ˚C				10 ˚C				22 ˚C			
	River	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*	Summer	*(Std Err)*	Winter	*(Std Err)*
Chalk	Ebble	8.09	*7.15*	5.68	*4.80*	11.43	*9.07*	3.21	*1.28*	61.29	*30.84*	30.72	*12.81*
	Wylye	0.75	*0.31*	7.22	*2.00*	1.09	*0.38*	8.87	*4.38*	21.35	*5.73*	61.96	*13.11*
	Wylye Patch A	45.89	*20.53*	326.87	*62.51*	90.65	*41.64*	515.68	*107.35*	482.87	*136.10*	1889.84	*354.49*
	Wylye Patch B	2.99	*2.79*	28.46	*11.32*	0.21	*0.14*	44.03	*15.66*	28.81	*28.04*	269.84	*98.69*
	Rushall-chalk	0.99	*0.23*	5.76	*5.64*	1.61	*0.56*	0.31	*0.16*	117.17	*51.91*	43.68	*41.60*
Sand	Avon	0.17	*0.08*	0.01	*0.00*	0.06	*0.01*	0.01	*0.00*	1.23	*0.39*	0.01	*0.01*
	Avon Patch A	0.50	*0.43*	NA	*NA*	0.10	*0.05*	NA	*NA*	4.89	*4.31*	NA	*NA*
	Avon Patch B	3.20	*3.05*	0.07	*0.05*	0.26	*0.11*	0.28	*0.18*	3.72	*3.17*	0.50	*0.12*
	Nadder	0.35	*0.25*	0.31	*0.29*	0.34	*0.24*	0.13	*0.08*	2.48	*1.52*	1.49	*0.97*
	Rushall-sand	1.23	*0.24*	NA	*NA*	1.42	*0.51*	NA	*NA*	15.69	*3.03*	NA	*NA*
Clay	Priors	2.51	*1.38*	6.63	*3.38*	1.36	*0.50*	4.85	*2.82*	6.93	*1.80*	23.04	*16.94*
	Sem	1.17	*0.76*	7.89	*7.70*	0.50	*0.21*	4.79	*3.95*	2.68	*0.77*	22.33	*21.02*
	Cools	3.36	*1.85*	30.43	*9.71*	2.70	*0.93*	72.18	*26.66*	73.79	*40.87*	134.82	*56.33*

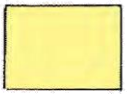
Table 7.2: Rate of potential production of methane in the nine study rivers in summer and winter. Results are averages (*n = 5*), of each of the three temperatures incubations were conducted at.

7.2 Mapping of Sites

The following pages show the maps generated and used to calculate percentage cover of different sediment types, using the methods described in Section 4.2.3. These results are summarised in Figure 4.3.

Mapping Key


Gravel


Sand | Fine Sediment

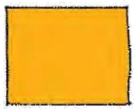

Sandy | Fine Sidebar


Clay

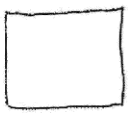

Clay | Woody

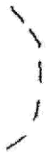

Submerged Vegetation (eg Ranunculus)


Emergent Vegetation (eg Reeds)


Cobbles/Stones + Clay


Woody Debris

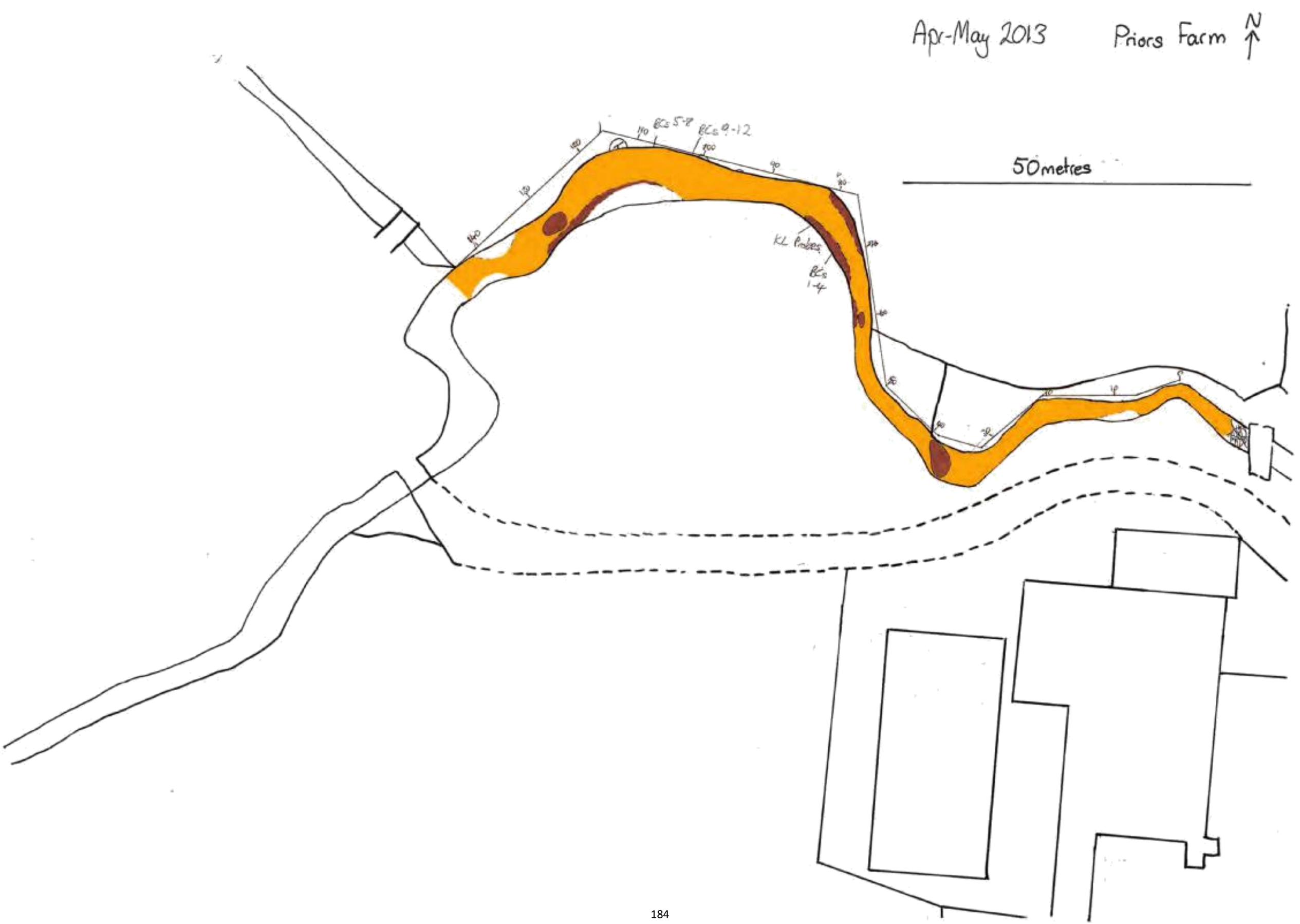

Exposed / Bank


Sidebar

Overhanging Tree


Piezometers

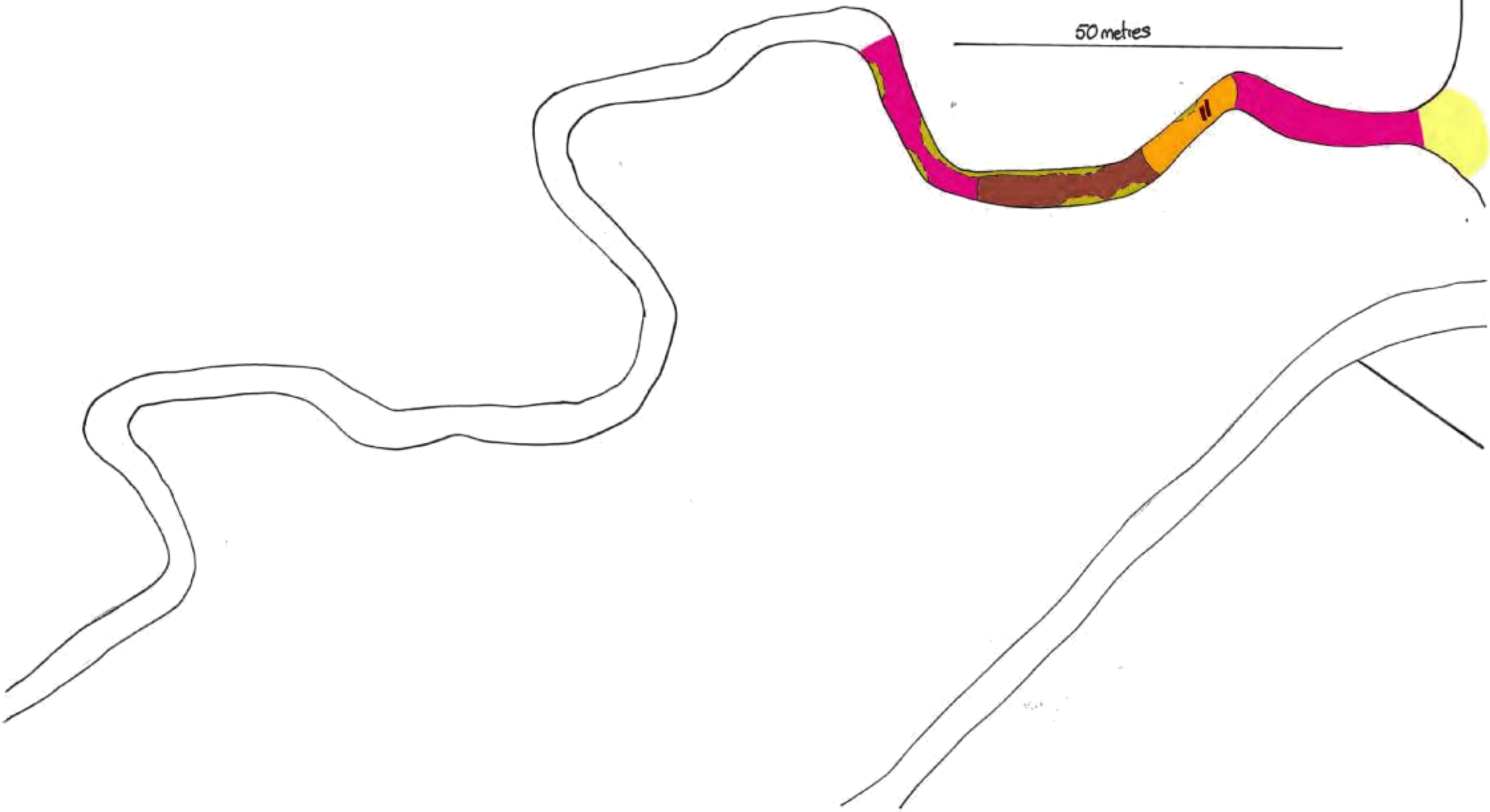
Apr-May 2013 Priors Farm N



Apr-May 2013 Sem

N

50 metres



Apr-May 2013

Ebble

N

50metres

KL Probes

BC: 1-4

→ BC: 5-12

Apr-May 2013
Wylye

N

50 metres

BCs 9-12

BCs 5-8

K-L Pnteas


Marden
N
50 metres
Apr-May 14
40
PCs 1-4
30
KL Probes
50
60
30
20
10
0
PCs 5-8

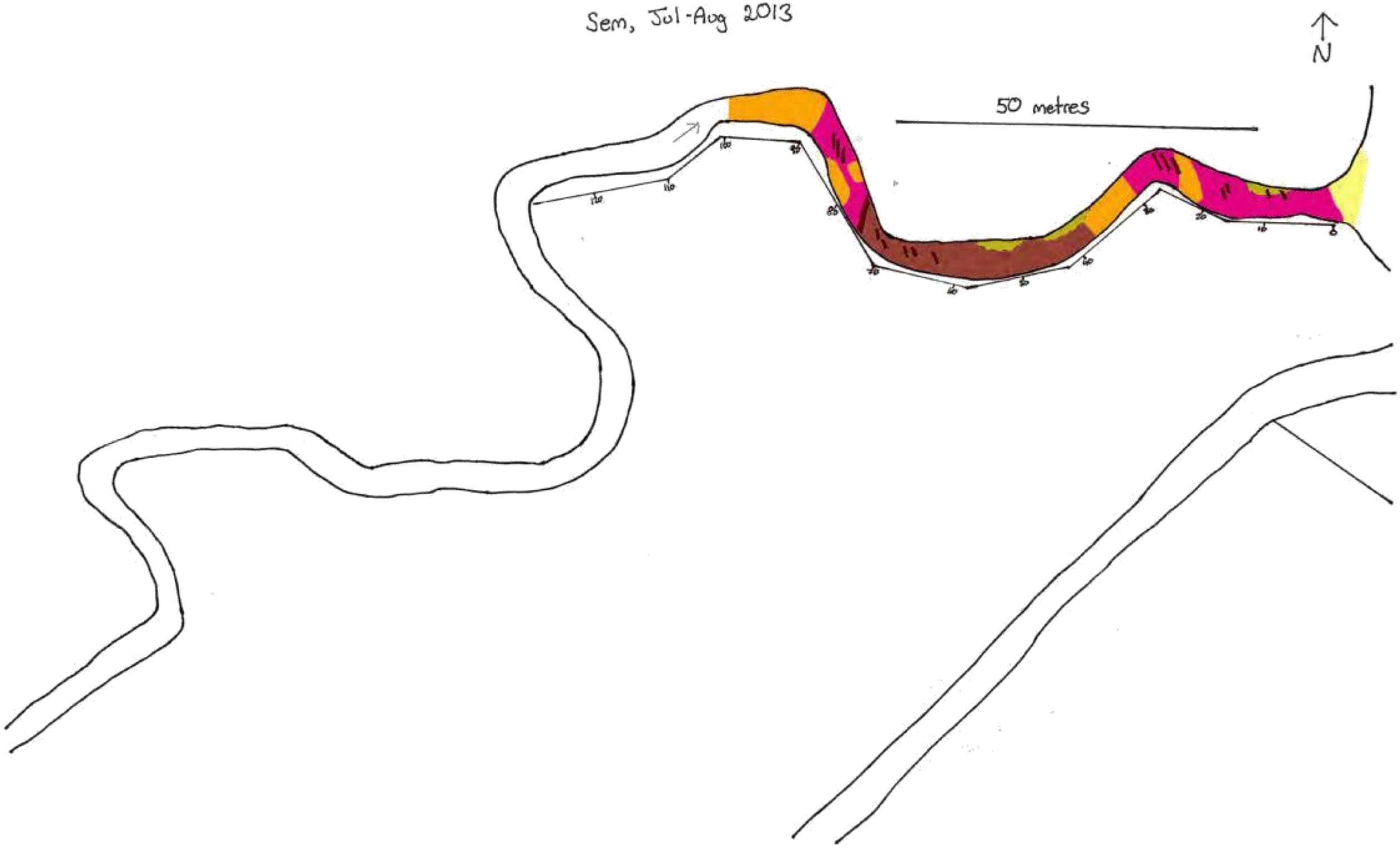

Nadder → N
Apr-May 13
50 metres
150
145
140
135
130
125
115
105
100
90
80
70
60
50
40
30
20
10
0

Priors Farm (Sem Trib) Jul-Aug 2013

N

50 metres

Sem, Jul-Aug 2013



Ebble, Jul-Aug 2013

N

50 metres

Wylye, Jul-Aug 2013

N

50 meters

Marden (West Avon) Jul-Aug 2013

50metres

N

0

10

20

30

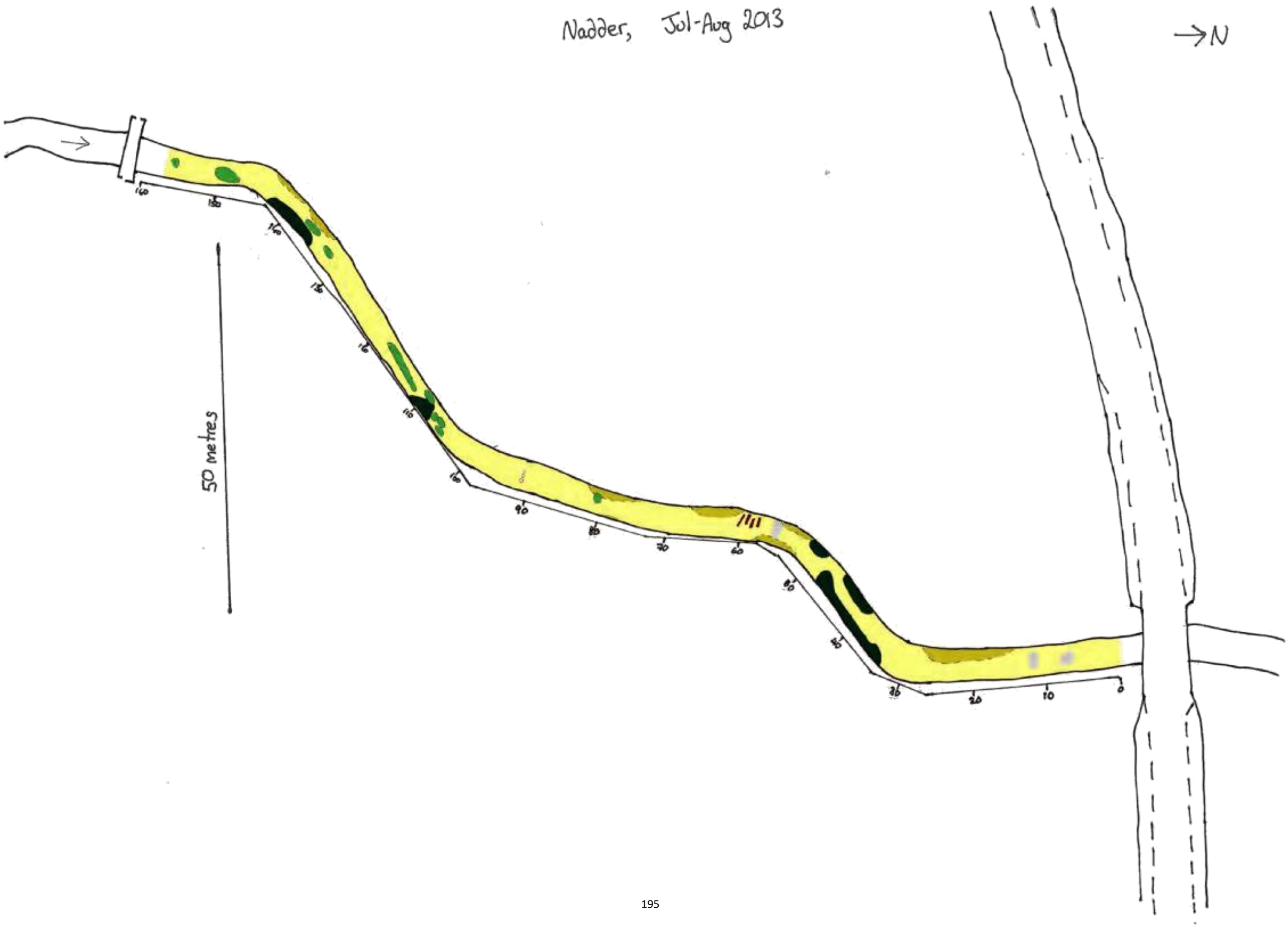
40

50

60

70

Nadder, Jul-Aug 2013



Priors Farm, Oct-Nov 2013

N

50 m

Ebble, Oct-Nov 2013

N

50m

Dead ranunculus + fine sed

Emergent gravel

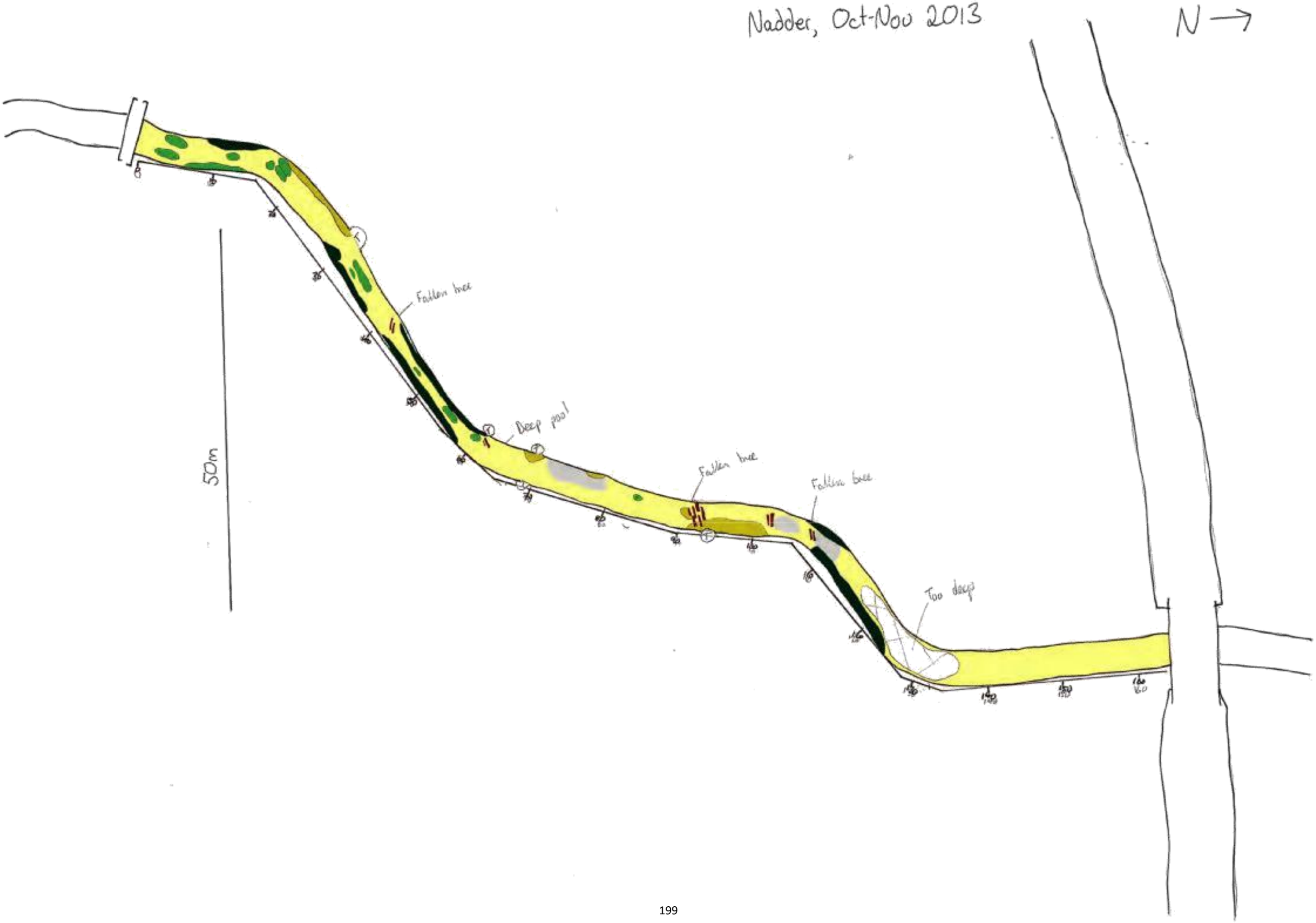
Wylye, Oct-Nov 2013

N

50m

Narrowed banks

Nadder, Oct-Nov 2013

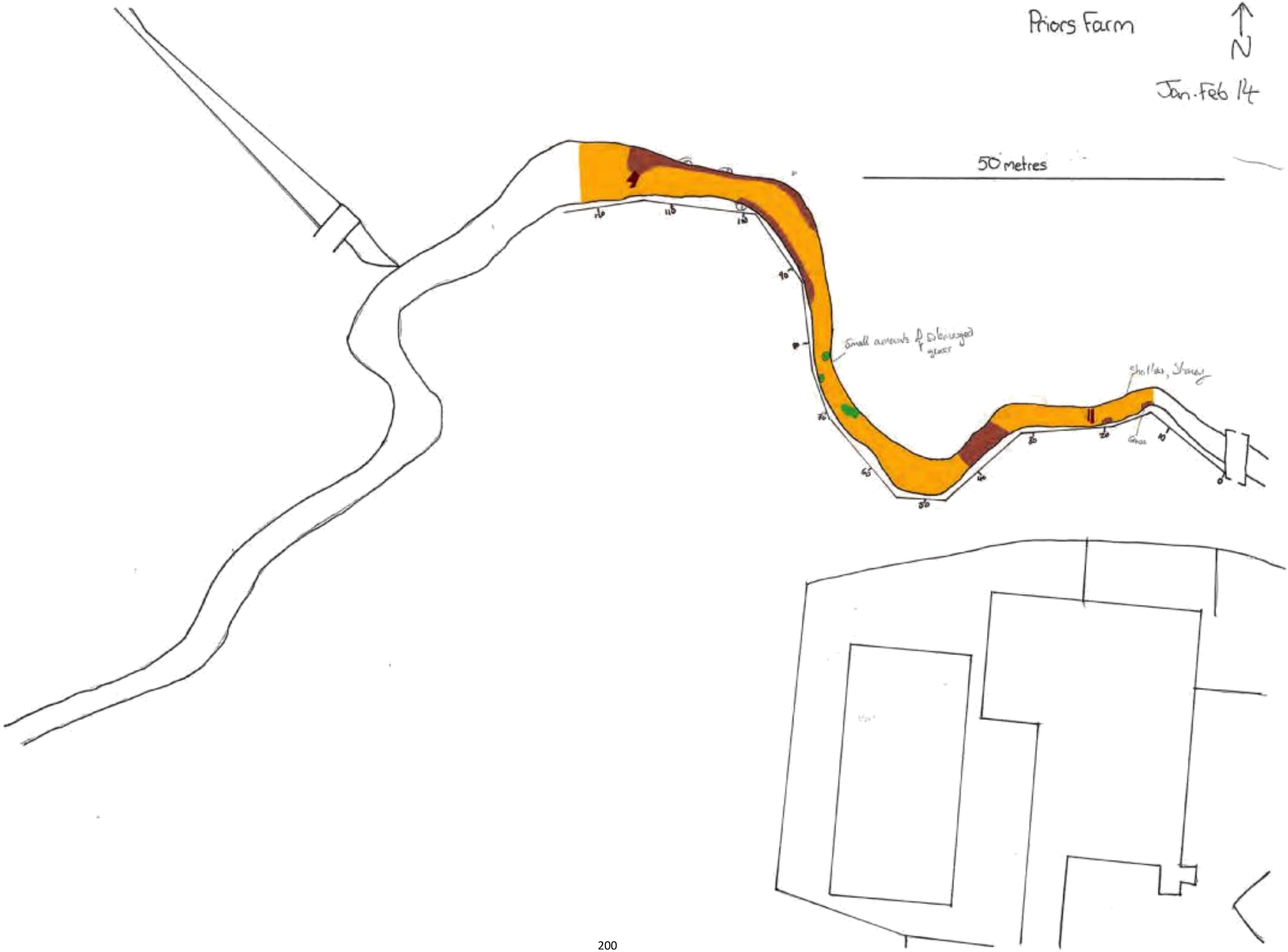


Priors Farm

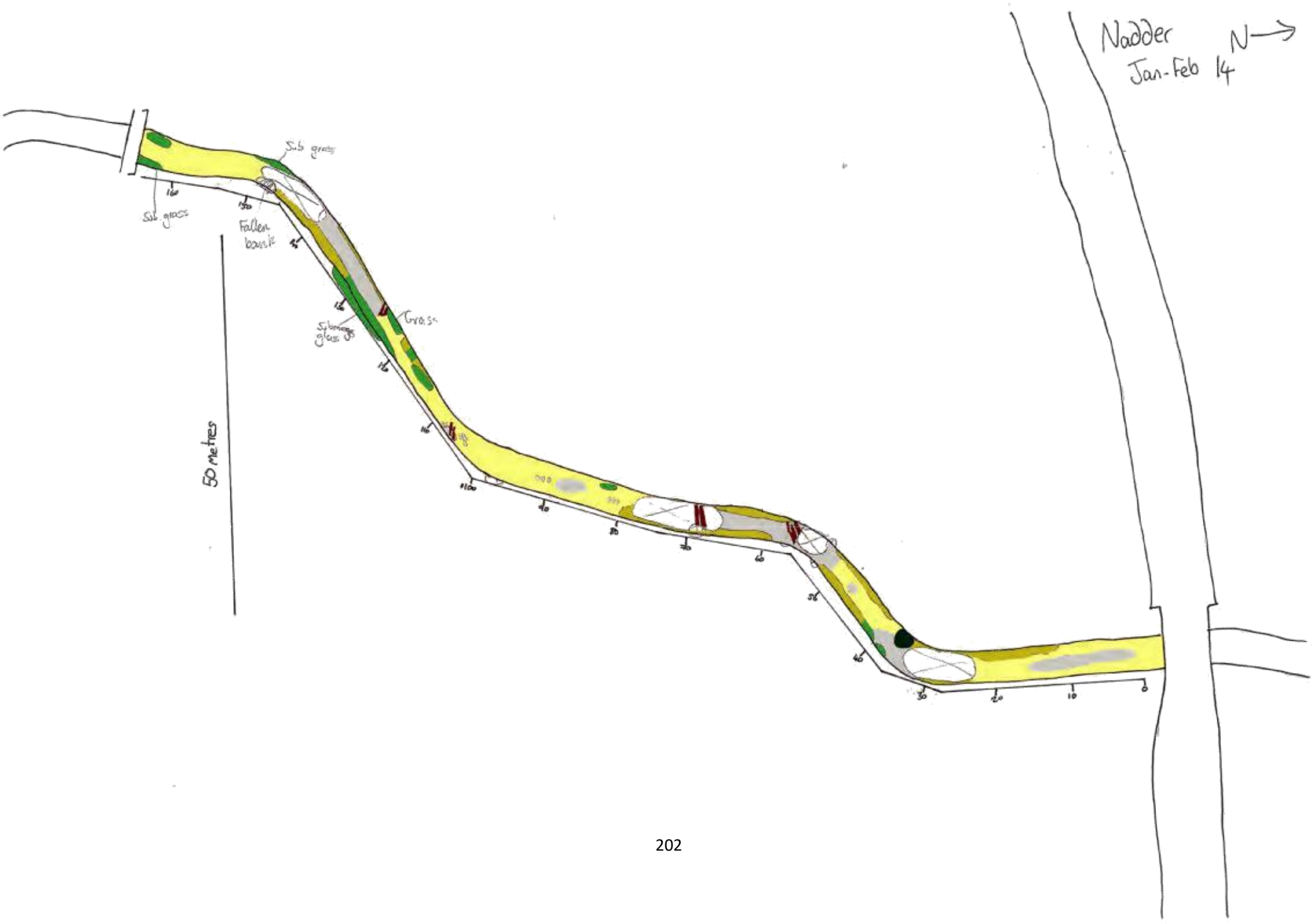
N

Jan. Feb 14

50 metres




Wylye
Jan-Feb 14
50 metres


Nadder
N
Jan-Feb 14
Sub grass
Sub. grass
Fallen branch
Grass
50 metres